The leaf essential oil of *Abies grandis* (Doug. ex D. Don) Lindl. *(Pinaceae)*: revisited 38 years later

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA Robert_Adams@baylor.edu

Michael Kauffmann
Biology Department, Humboldt State University, 4312 Lentell Road, Eureka, CA 95503

and

Frank Callahan
P. O. Box 5531, Central Point, OR 97502

ABSTRACT

The first report on the volatile leaf terpenoids of *Abies grandis* was in 1976 by Ernst von Rudloff. Using new GCMS technology that has been developed in the interim, a comprehensive analysis was performed on the leaf terpenoids of *A. grandis* and is presented. The oil of *A. grandis* is dominated by β-pinene (20.3 - 31%), bornyl acetate (12.7 - 26.2%), β-phellandrene (13.7 - 25.2%) and camphene (8.3 - 11.5%), with moderate amounts of α-pinene (4.4 - 7.4%), α-terpinene (1.1 - 2.2%), terpinolene (1.3 - 2.9%) and α-terpineol (1.1 - 3.6%). Fifty nine compounds were identified encompassing monoterpenes, sesquiterpenes and one diterpene (abietadiene). Only 1-epi-cubenol appeared to differ between coastal (0.5 - 0.7%) and inland grand fir (0 - trace). Otherwise, just as von Rudloff (1976) and Zavarin et al. (1977) concluded, the volatile oils (leaf and wood) do not differentiate coastal and inland grand fir. The von Rudloff (1976) analysis, using packed GC columns, agreed closely with the present analysis.
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KEY WORDS: *Abies grandis,* terpenes, composition, volatile leaf oils, Pinaceae.

In 1976, I (RPA) was on a sabbatical with Ernst von Rudloff in Saskatoon, Canada when his paper on the volatile leaf oil of grand fir (*Abies grandis*) was published (von Rudloff, 1976). As was his usual procedure, he reported on within- and between-tree variation before reporting on inland and coastal populations. von Rudloff (1976) reported quantitative data for thirteen monoterpenes, alcohols and acetates, with combined values for the sesquiterpene hydrocarbons and alcohols using a packed GC column. This was before the widespread use of fused-quartz capillary columns. My (RPA) work developing a computerized mass spectra library of volatile oil components was began in von Rudloff's lab and with the gracious sharing of his large stock of known terpenoids (Adams, et al. 1979; Adams, 2007). After 38 years of progress in the identification of terpenoids, those days of trapping individual components from packed columns and then running IR and NMR, pale in comparison to present-day technology enabling one to run GCMS and identify 95 - 98% of more than 100 components in two hours.

von Rudloff (1975, 1976) reported only minor differences in the volatile leaf oils between inland and coastal *A. grandis* and turned his attention to analysis of other conifer oils. Zavarin et al. (1977) examined quantitative variation in eight monoterpenes in wood oleoresin in 395 samples from 48 populations of *A. grandis*. They concluded "..noteworthy is the absence of any difference between coastal and Rocky Mountain populations...". The reports from von Rudloff and Zavarin's labs, appear to have deterred any additional research on the leaf essential oils of *A. grandis*. A search of the literature revealed only two more recent papers on the leaf oils of *A. grandis*. Muzika et al. (1989) examined the effects of nitrogen fertilization on 10 leaf terpenes and Muzika et al. (1990) reported a comparison of extraction methods on yields of monoterpenes from *A. grandis*.

This paper, using high resolution capillary GCMS, presents the first detailed report on the composition of the volatile leaf oil of *A. grandis* since the original report of von Rudloff (1976).

MATERIALS AND METHODS

Leaf samples were collected from *Abies grandis,* Kane Creek, OR, 42° 21' 39" N, 123° 01' 16" W, 2268 ft., *Adams (F. Callahan) 14343-14347*; 14 mi e of Coeur d'Alene, ID, on I90, 47° 37' 16" N, 116° 31' 10" w, 3100 ft., *Adams 14363-14364*; Mt. Spokane Park, WA, 47° 54' 13" N, 117° 06' 09" W, 4528 ft., *Adams 14358-14362*; 3 mi. se of Sequim, WA, 48° 2.08" N, 123° 1.36" W, 72 ft., *Adams 12913-12917*; Del Norte Co., CA, 41° 45' 27.99" N, 124° 08' 41.84" W, 289 ft., *Adams (M. Kauffmann) 12970-12974*, Humboldt Co., CA, 40° 45' 57.95" N, 124° 07' 18.31" W, 310 ft., *Adams (M. Kauffmann) 12975-12979*. Voucher specimens are deposited in the herbarium, Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The leaf oil of *Abies grandis* was clear in color. The oil of *A. grandis* (Table 1) is dominated by β-pinene (20.3 - 31%), bornyl acetate (12.7 - 26.2%), β-phellandrene (13.7 - 25.2%) and camphene (8.3 - 11.5%), with moderate amounts of α-pinene (4.4 - 7.4%), α-terpinene (1.1 - 2.2%), terpinolene (1.3 - 2.9%) and α-terpineol (1.1 - 3.6%). Fifty nine compounds were identified encompassing monoterpenes, sesquiterpenes and diterpenes (Table 1). Only 1-epi-cubenol (Table 1) appeared to differ between coastal (0.5 - 0.7%) and inland grand fir (0 - trace). Otherwise, just as von Rudloff (1976) and Zavarin et al. (1977) concluded, the volatile oils (leaf and wood) do not differentiate coastal and inland grand fir. The von Rudloff (1976) analysis, using packed GC columns thirty eight years ago, agreed closely with the present analysis (Table 1).

ACKNOWLEDGEMENTS

The senior author (RPA) owes a debt of gratitude to Ernst von Rudloff who came to the University of Texas in Austin in 1967 and taught me about steam distillation of conifer oils, gas chromatography and the identification of terpenoids. In 1976, Ernst hosted me on a sabbatical in his lab at the Prairie Regional Lab of the Canada Natural Research Council, Saskatoon, Saskatchewan. Ernst was always encouraging and modeled a thorough scientific approach to research. I count him as both a mentor and a friend. This research supported with funds from Baylor University.

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Table 1. Comparison of leaf oil compositions of coastal and inland *Abies grandis* populations. Only 1-epi-cubenol (in bold face) appears to separate coastal and inland groups. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index based a linear calculation on DB-5 column. Sqm,WA = Sequim, WA; sw OR = sw of Central Point, OR; Hmb, CA = Humboldt Co. CA; CdA, ID = Coeur d'Alene, ID. The data for V.I., BC (Vancouver Island, BC, EvR 1976) and CF,ID (Clark Fork, ID, EvR 1976) were taken from von Rudloff (1976, Table 3),

		Coastal				inland	
		2014 collections			1976	1976	2014
KI	compound	Sqm,WA	sw OR	HMB,CA	V.I.,BC	C.F.,ID	CdA,ID
846	(E)-hexenal	0.3	0.2	0.1			0.3
884	santene	t	t	0.3			0.2
921	tricyclene	0.6	0.4	1.0	1.3	1.2	0.7
924	α-thujene	0.4	t	t			0.1
932	α-pinene	4.7	5.0	7.4	6.0	8.1	4.4
946	camphene	11.5	8.3	10.5	13.8	14.0	10.7
969	sabinene	t	t	t			t
974	β-pinene	30.8	31.1	20.3	21.4	25.2	20.3
988	myrcene	1.0	1.4	1.0	1.0	1.1	0.9
1002	α-phellandrene	1.0	1.9	0.8			0.7
1008	δ-3-carene	0.1	t	0.5			0.7
1014	α-terpinene	2.0	2.1	1.4			1.1
1020	p-cymene	0.2	t	t			t
1024	limonene	1.1	0.8	1.3	2.0	2.5	2.1
1025	β-phellandrene	13.7	15.9	25.2	11.3	16.0	18.9
1032	(Z)-β-ocimene	t	t	-			t
1054	γ-terpinene	0.7	0.6	0.4			0.3
1086	terpinolene	2.9	2.8	1.3			1.3
1095	linalool	t	t	t			t
1114	endo-fenchol	0.3	-	t			0.3
1118	cis-p-menth-2-en-1-ol	0.2	t	0.2			t
1122	α-campholenal	0.2	t	t			t
1136	trans-p-menth-2-en-1-ol	0.4	t	0.2			t
1141	camphor	0.4	0.3	0.3	0.7	0.6	0.5
1145	camphene hydrate	t	0.2	0.1			0.3
1148	citronellal	0.5	0.4	0.6			0.4
1148	iso-isopulegol	t	t	0.2			t
1165	borneol	0.4	0.2	0.5	0.7	0.5	0.2
1174	terpinen-4-ol	0.2	0.2	0.2			0.2
1186	α-terpineol	3.6	2.5	1.1	0.5	0.5	1.8
1195	cis-piperitol	-	-	t			-
1207	trans-piperitol	-	-	t			-
1223	citronellol	0.3	0.7	1.0			0.4
1264	geranial	-	t	-			t
1287	bornyl acetate	17.0	12.7	17.6	18.5	18.8	26.2
1298	2-undecanone	t	t	t			t
1345	α-cubebene	t	t	t			t
1350	citronellyl acetate	0.2	0.9	0.5	0.9	0.6	0.2
1374	α-copaene	t	t	0.1			t
1379	geranyl acetate	1.2	0.3	0.4	0.3	0.7	0.3

Table 1 (contd.)

		Coastal				inland	
		2014 collections			1976	1976	2014
KI	compound	Sqm,WA	sw OR	HMB,CA	V.I.,BC	C.F.,ID	CdA,ID
1417	(E)-caryophyllene	t	t	t			t
1451	trans-muurola-3,5-diene	t	t	t			t
1475	trans-cadina-1(6),4-diene	t	0.3	t			0.1
1493	trans-muurola-4(14),5-diene	0.2	0.6	0.2			0.2
1493	epi-cubebol	0.2	0.2	0.3			0.3
1500	α-muurolene	0.2	0.2	0.1			t
1514	cubebol	0.3	0.6	0.5			0.6
1522	δ-cadinene	0.9	1.2	0.7			1.0
1528	zonarene	t	0.2	t			t
1533	trans-cadina-1,4-diene	0.1	0.4	0.1			0.2
1561	(E)-nerolidol	t	t	t			t
1614	(selina-6-en-4α-ol)	t	0.2	0.1			t
1627	**1-epi-cubenol**	**0.6**	**0.7**	**0.5**			**t**
1645	cubenol	0.4	0.5	t			0.6
1644	α-muurolol	t	t	t			t
1652	α-cadinol	0.2	0.3	t			0.3
1665	intermedeol	-	1.2	-			-
1722	(2Z,6E)-farnesol	-	t	-			t
1864	benzyl salicylate	-	0.1	-			-
2087	abietadiene	-	-	0.1			-

Juniperus communis in Azerbaijan: analyses of nrDNA and cpDNA regions

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
Robert_Adams@baylor.edu

Vahid Farzaliyev
Deputy Director for Science, Central Botanical Garden, Azerbaijan National Academy of Science,
Badamdar shosse 40, Baku, Azerbaijan, AZ 1073.

Alexander N. Tashev
University of Forestry, Dept. of Dendrology
10, Kliment Ochridsky Blvd., 1756 Sofia, Bulgaria

and

Andrea E. Schwarzbach
Department of Biomedicine, University of Texas at Brownsville, Brownsville, TX 78520, USA.

ABSTRACT

Juniperus 'pygmaea' from Azerbaijan was analyzed by DNA sequence data from nrDNA plus four cp DNA regions (4315 bp) and found in a clade with *J. communis 'oblonga'* (= *J. communis*) Armenia, not with *J. c.* forma *pygmaea* of Bulgaria. It seems prudent to not recognize this variant taxonomically but treat it as *J. communis*. Published on-line **www.phytologia.org** *Phytologia 97(1): 6-11 (Jan 2, 2015).* ISSN 030319430.
KEY WORDS: *Juniperus communis* forma *pygmaea, J. communis, J. oblonga, J. pygmaea,* Azerbaijan, nrDNA, cpDNA sequences, taxonomy.

Several taxa closely related to *Juniperus communis* are currently recognized (Askerov, 2005; Prilipko, 1961) in Azerbaijan (= nomenclature of Adams, 2014): *J. hemispherica* J. & C. Presl. (= *J. communis* var. *hemispherica* [J. & C. Presl.] Parl.); *J. oblonga* M.-Bieb. (= *J. communis* L.) and *J. pygmaea* M.-Bieb. In Azerbaijan, *Juniperus 'pygmaea'* grows as an upright to spreading shrub.

Recently, Adams and Tashev (2013) compared the leaf essential oils of *J. communis, J. pygmaea* and *J. sibirica* from Bulgaria with the oils of *J. communis* of Sweden and *J. saxatilis* of Switzerland. From their analysis, the oils do not ordinate *J. communis, J. pygmaea* and *J. sibirica* from Bulgaria into separate groups, but they are generally interspersed. Additional research (Adams, Tashev and Schwarzbach, 2014) using DNA sequences from nrDNA and four cp regions gave no clear separation of *'pygmaea'* from *J. communis* and *J. c.* var. *saxatilis*. They concluded that the shrubby habit is likely controlled by only a few genes and recognized the taxon as *J. communis* f. *pygmaea* (K. Koch) R. P. Adams and A. N. Tashev.

The leaves and seed cones of *J. 'pygmaea'* of Azerbaijan are quite similar to those of *J. c.* f. *pygmaea* of Bulgaria and *J. c.* var. *oblonga* of Armenia (Fig. 1).

Figure 1. Leaves and seed cones of *J. c.* f. *pygmaea,* Bulgaria *J. c.* f. *'pygmaea',* Azerbaijan and *J. c.* var. *oblonga*, Armenia.

The purpose of this study was to compare data from nrDNA and four cpDNA regions of *J. pygmaea* from Azerbaijan with other members of *Juniperus* sect. *Juniperus* from the eastern hemisphere to determine the taxonomic affinity of *J. 'pygmaea'* from Azerbaijan.

MATERIALS AND METHODS

Plant material - Bulgaria, *J. communis* var. *communis*, *Adams Lab acc 13730-31, 14058-60, (Alex Tashev, 2012-JC1-5)*, Eastern Rhodopes, in protected site "Gumurdjinsky Shezhnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca,* 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m. *J. communis* f. *pygmaea*, *Adams Lab acc. 13734-35, 14064-66,(Alex Tashev, 2012-JP1-5)*, Central Rhodopes. Mursalitza part, locality "Piramidata". On high-mountain meadow, on a limestone rock near a forest of *Pinus sylvestris* together with *Picea abies,* 41° 40' 22.8" N; 24° 26' 36.6" E. elev. 1756 m.
J. communis var. *saxatilis* - Bulgaria, *Adams Lab Acc. 13732-33, 14061-63,(Alex Tashev, 2012-JSI1-5),* Vitosha Region. Nature Park "Vitosha". Above the hut "Aleco" near the alpine timber line formed by a forest of *Picea abies*. On silicate rock together with *Vaccinium myrtillus, V. uliginosum, Ribes petraeum, Rubus idaeus, Calamagrostis arundinacea, Festuca valida (*Bulgarian endemic*),* 42° 34' 52.1" N; 23° 17' 28.0" E. elev. 1848 m.
J. 'pygmaea' - Azerbaijan, shrubs, 0.5 - 1m tall, with *J. sabina*, on rocks in mountains. 41° 11.790' N; 48° 15.313' E. elev. 1649m *Adams Lab acc. 14321-14325* (*V. Farzaliyev 1-5*) 6 Jun 2014.
Exemplar specimens: *J. communis* var. *communis,* Stockholm, Sweden, *Adams 8167 (7846-7848); J. communis* var. *saxatilis,* Switzerland, *Adams 11164 (7618-7621)*. Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from http://www.geneious.com/) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v. 3.1 (Ronquist and Huelsenbeck, 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al. ,2009; Adams, 1975; Veldman, 1967).

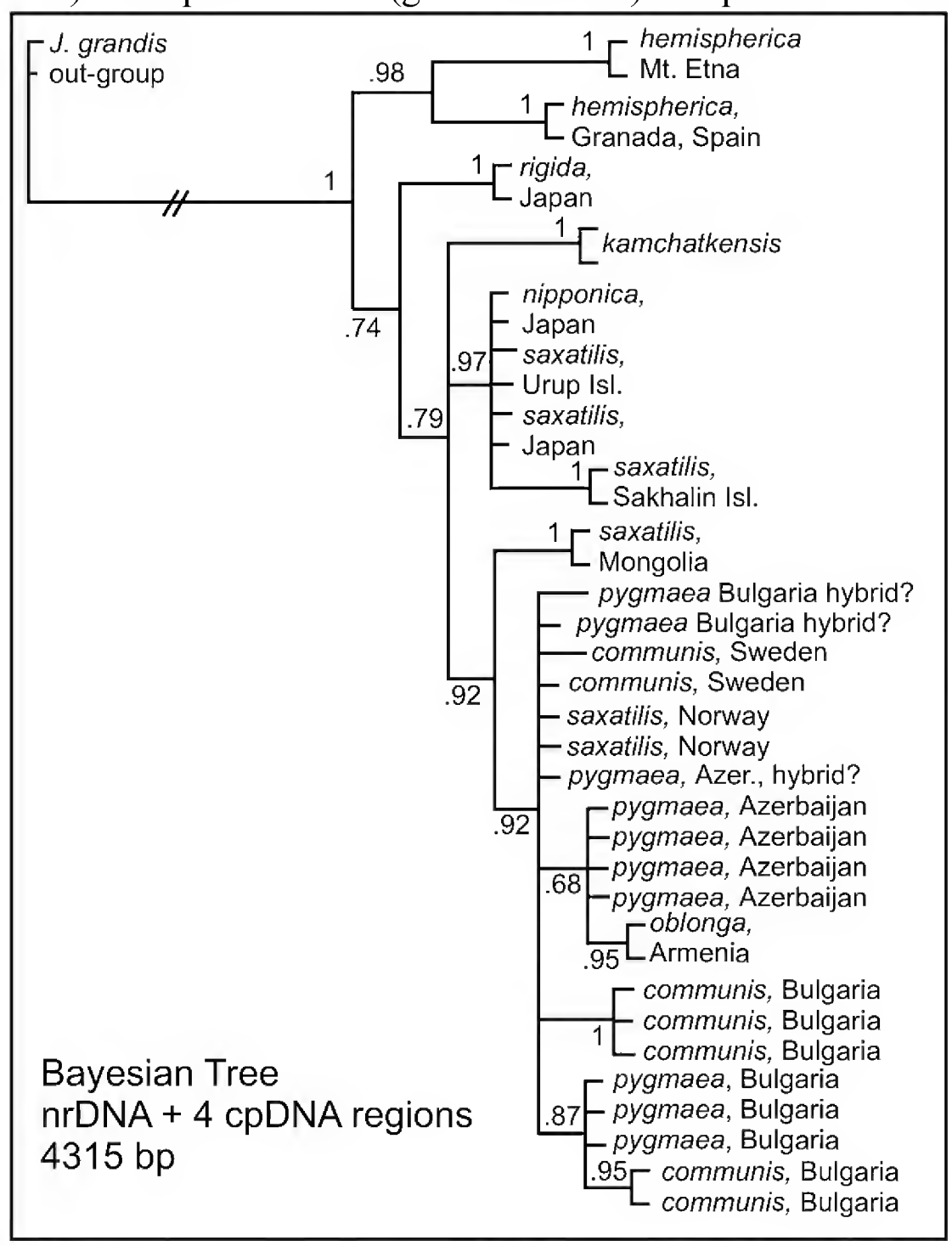

Figure 2. Bayesian tree of *Juniperus* sect. *Juniperus* taxa of the eastern hemisphere. Numbers at branch points are posterior probabilities. See text for discussion.

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) and four cp-regions (petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF) yielded 4315 bp of data. The Bayesian consensus tree (Fig. 2) revealed that four of the *J. 'pygmaea'* of Azerbaijan, are in a clade with *J. communis* '*oblonga*' of Armenia (= *J. communis*, Adams 2014). The fifth *J. 'pygmaea'* was polymorphic for two bp in its nrDNA and may be a hybrid.

The *J. 'pygmaea'* plants of Azerbaijan are not in a clade with typical *J. communis* f. *pygmaea* of Bulgaria.

To examine the magnitude of the differences, a minimum spanning network was constructed (Fig. 3). *Juniperus communis*, eastern hemisphere, is divided into three groups: *J. communis*, Europe, *J. communis*, Japan and far east, and *J. c.* var. *hemispherica*, the latter divided among Mt. Etna, Sicily (type locality) and Sierra Nevada, Granada, Spain. All the samples of *J. 'pygmaea'* of Azerbaijan, are tightly grouped with *J. communis* from Europe (Fig. 3). Interestingly, the *J. communis 'oblonga'* of Armenia differs by 3 MEs (indels in this case) from *J. communis* of Sweden. Because the two polymorphic bp were removed from the nrDNA of the *J. 'pygmaea'*, these five samples show no variation. The two samples of *J. communis* f. *pygmaea* of Bulgaria, next to *J. communis* of Sweden in Fig. 3, appear to be hybrids.

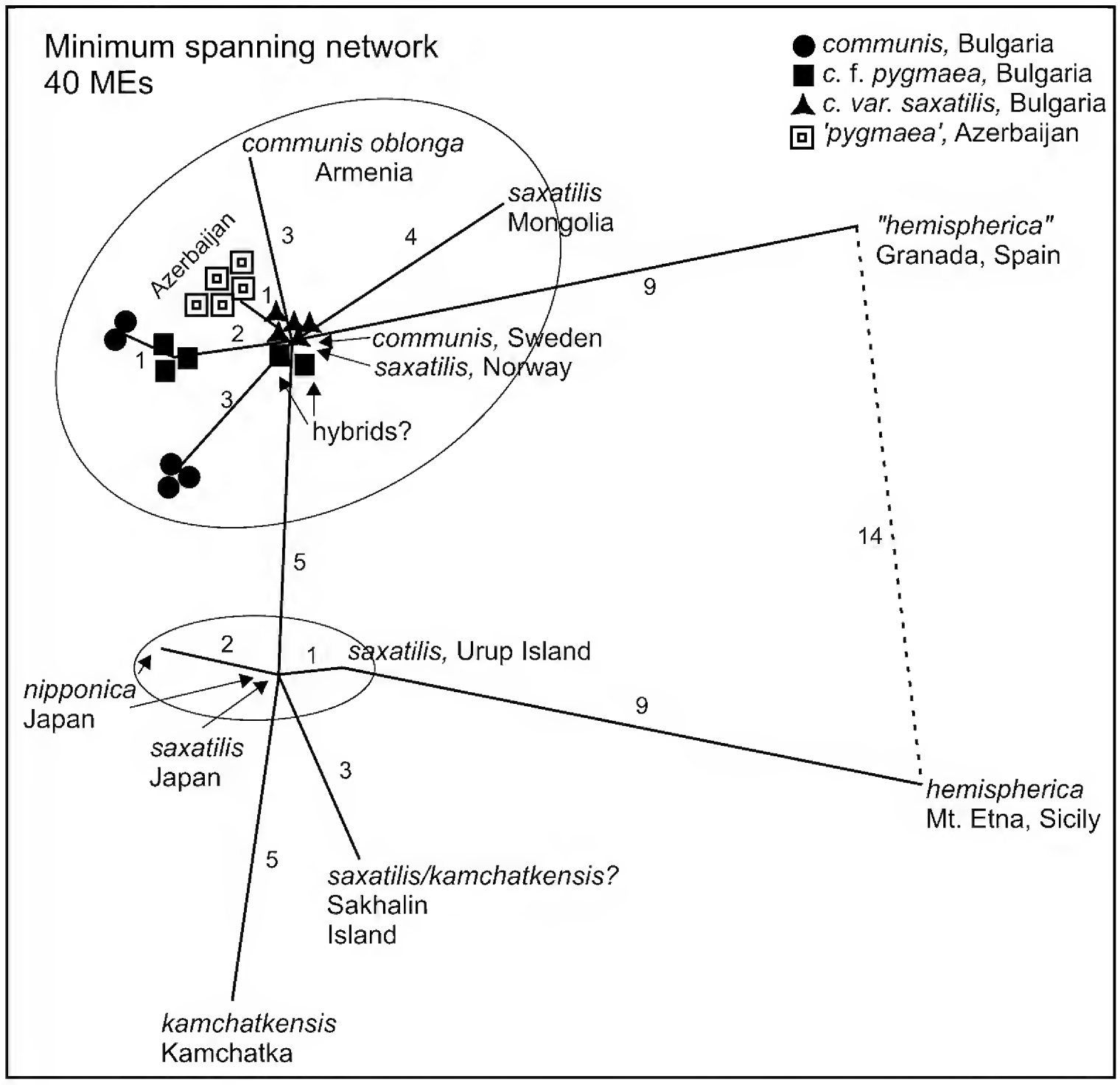

Figure 3. Minimum spanning network of *J. communis* and its varieties based on 40 MEs (mutational events = SNPs + indels). Numbers next to the links are the number of MEs. The dashed line is the second shortest link between the *hemispherica* taxa.

Examination of the habits of *J. communis* f. *pygmaea* of Bulgaria, *J. communis 'oblonga'* of Armenia compared to *J. 'pygmaea'* of Azerbaijan (Fig. 4) is useful. The habit of *J c.* f. *pygmaea* of Bulgaria, is a compact shrub with rigid branchlets. In contrast, *J. 'pygmaea'* of Azerbaijan, is a large, open shrub with weeping branchlets and is quite similar to *J. communis 'oblonga'* of Armenia. *Juniperus 'pygmaea'* differs from *J. communis* in Spain and Hungary in having weeping, versus erect, foliage (Fig. 4). However, the flaccid (weeping) branchlets may be controlled by only a few genes (as suggested by the lack of DNA differences). The wide range of plant habits is shown in the Hungary population of *J. communis* (Fig. 4) with upright trees, shrub-trees, and shrubs as apical growth is differentially expressed.

In summary, *J. 'pygmaea'* of Azerbaijan, is the same taxon as *J. communis 'oblonga'* of Armenia and not *J. communis* f. *pygmaea* of Bulgaria. It should be treated at *J. communis* in Azerbaijan.

Figure 4. Plant habits of *J communis* f. *pygmaea*, Bulgaria, *J. communis 'oblonga',* Armenia compared to *J. 'pygmaea'*, Azerbaijan and *J. communis*, Spain and Hungary.

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Taxonomic study of the *Tithonia calva* complex (Asteraceae, Heliantheae)

Billie L. Turner
Plant Resources Center
The University of Texas
Austin TX 78712

ABSTRACT

The *Tithonia calva* Sch.-Bip. complex has been variously treated, some workers recognizing 2 species under its fabric, and yet others accepting but 2 or 3 varieties. The taxonomy of the complex is reviewed and it is concluded that the group is best treated as containing 2 non-intergrading species: *T. calva*, occurring in southernmost Sinaloa and closely adjacent Durango, in pine forests from 1000-2000 m; and *T. auriculata*, occurring in oak forests from 100-1000 m. A key to the taxa is provided, along with maps showing their distribution. Published on-line **www.phytologia.org** *Phytologia 97(1): 12-15 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: Asteraceae, Heliantheae, *Tithonia*, *T. auriculata*, *T. calva*, Mexico

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Preoccupation with the Asteraceae of Mexico (Turner 1997, etc.) has occasioned the present paper.

Tithonia calva was treated by La Duke (1982) as the only member of sect. Mirasolia of **Tithonia,** this typified by material collected by Seemann in Sinaloa, Mexico, during the fall of 1849, presumably from pine forests along the route to the state of Durango, this perhaps paralleling Highway 40 of present-day maps.

Brandegee (1900) described *Gymnolomia auriculata* (typified by collections from Cofradia, Sinaloa), this transferred to **Tithonia** by Blake (1918); La Duke (1982) reduced the latter to varietal rank as *T. calva* var. *auriculata*, stating "Its closest relation is to *T. calva* var. *lancifolia*," the latter originally described as a variety of *Gymnolomia calva* by Robinson and Greenman, and typified by material from Acaponeta, Nayarit. Blake (1918) treated var. *lancifolia* as a subspecies of **T. calva**, while retaining **T. auriculata**.

La Duke (1982) subsequently recognized **T. calva** as having three infraspecific categories: **1**) var. *calva*; **2**) var. *auriculata*; and **3**) var. *lancifolia*. McVaugh (1984), in his Flora Nova-Galiciana, included var. *auriculata* within his concept of *T. c.* var. *lancifolia* (albeit with some reservation), having noted, aptly, that var. *auriculata*, what "with its almost linear, sessile leaves that are cordate-auriculate at base...is apparently not very different from var. *lancifolia*." McVaugh's treatment, and his perceptive comments that var. *lancifolia* is largely confined to open hillsides in oak or palm-oak forests, largely between 250-1000 meters while var. *calva* is a plant of pine-oak forests at higher elevations (1000- 2000 m), strongly suggests that only two meaningful taxonomic units make up the complex. After examination of numerous sheets from throughout the area concerned, I agree with McVaugh's assessment, but would treat the taxa as specifically distinct, since they occupy different ecological zones and do not appear to intergrade.

The following summarizes my interpretation of the **T. calva** complex:

Key to taxa

1. Leaves mostly 1-2(3) cm wide; heads mostly 3-5(6) cm across the extended rays; middle and upper stems variously pubescent, the hairs mostly 2 mm long or less; plants mostly of oak forests, 100-1000 m.....**T. auriculata**
1. Leaves mostly 4-7 cm wide; heads (4) 5-8 cm across the extended rays; middle and upper stems pubescent with elongate, silky, trichomes 5-6 mm long; plants mostly of pine forests, 1000-2000 m.....**T. calva**

TITHONIA AURICULATA (T.S. Brandegee) S.F. Blake, Contr. Gray Herb. 54: 9. 1918.
Gymnolomia calva var. *lancifolia* Rob. & Greenm. 1899
Gymnolomia auriculata T.S. Brandegee 1905
Tithonia calva subsp. *lancifolia* (Rob. & Greenm) S.F. Blake 1921
Tithonia calva var. *lancifolia* (Rob. & Greenm.) McVaugh 1972

s Son, Sin, Nay and Jal, mostly tropical deciduous forests, 200-1000 m; Aug-Apr.

McVaugh has provided an excellent account of the taxon, and such need not be repeated here.

If treated as a variety, the correct name for this taxon would be *T. c.* var. *lancifolia;* if treated as a species, as done here, the correct name, under the present Code of Botanical Nomenclature, is **T. auriculata**. As noted above, the two taxa, as circumscribed by both McVaugh and myself, do not intergrade in regions of near contact, hence my specific bestowal. **Map 1**

This taxon is much more variable than **T. calva**, both in vegetative and floral features. Plants having relative broad leaves were treated as var. *lancifolia* by La Duke; at the same time he maintained var. *auriculata*. Indeed, a case might be made for the recognition of infraspecific taxa within my concept of **T. auriculata**, thus *Hernandez 654* from Mpio. de Elota, Sinaloa (TEX), has unusually pubescent leaves, these up to 2 cm wide, and stems with white villous hairs ca 2 mm long, but the overall gestalt is clearly that of **T. calva,** as interpreted here.

As noted above, nearly all of the collections of **T. auriculata** have been obtained in deciduous oak forests at elevations of 100-1000 meters, mostly along the Pacific frontal range as shown in Map 1; the seemingly disjunct nature of the populations concerned is likely to represented a gap in specimen acquisition, the area concerned having few roads of access.

TITHONIA CALVA Sch.-Bip. in Seemann, Bot. Voyage Herald. 305. 1856.
Mirasolia calva (Sch.-Bip. in Seemann) Benth & Hook. ex Hemsl. 1881
Gymnolomia calva (Sch.-Bip. in Seemann) A. Gray 1883

Chi, Sin, Dur and Nay, pine-oak woodlands, 1000-2000 m; Aug-Nov.

McVaugh (1984) has given an excellent description of the species, and this need not be repeated here. In addition to the characters used in the above key to distinguish between the two taxa, he noted, correctly, for **T. calva**, the thickened apical peduncles (2-6 mm across), and the mostly longer pales and larger disc florets. McVaugh also recognized the species as occurring only at high elevations of "S e Sin. and adjacent Dgo," much as I do, this contrary to the views of La Duke (1982). **Map 2**

ACKNOWLEDGEMENTS

Jana Kos edited the paper, for which I am grateful. Distribution maps are based upon specimens on file at LL-TEX, and upon specimens cited by McVaugh (1984).

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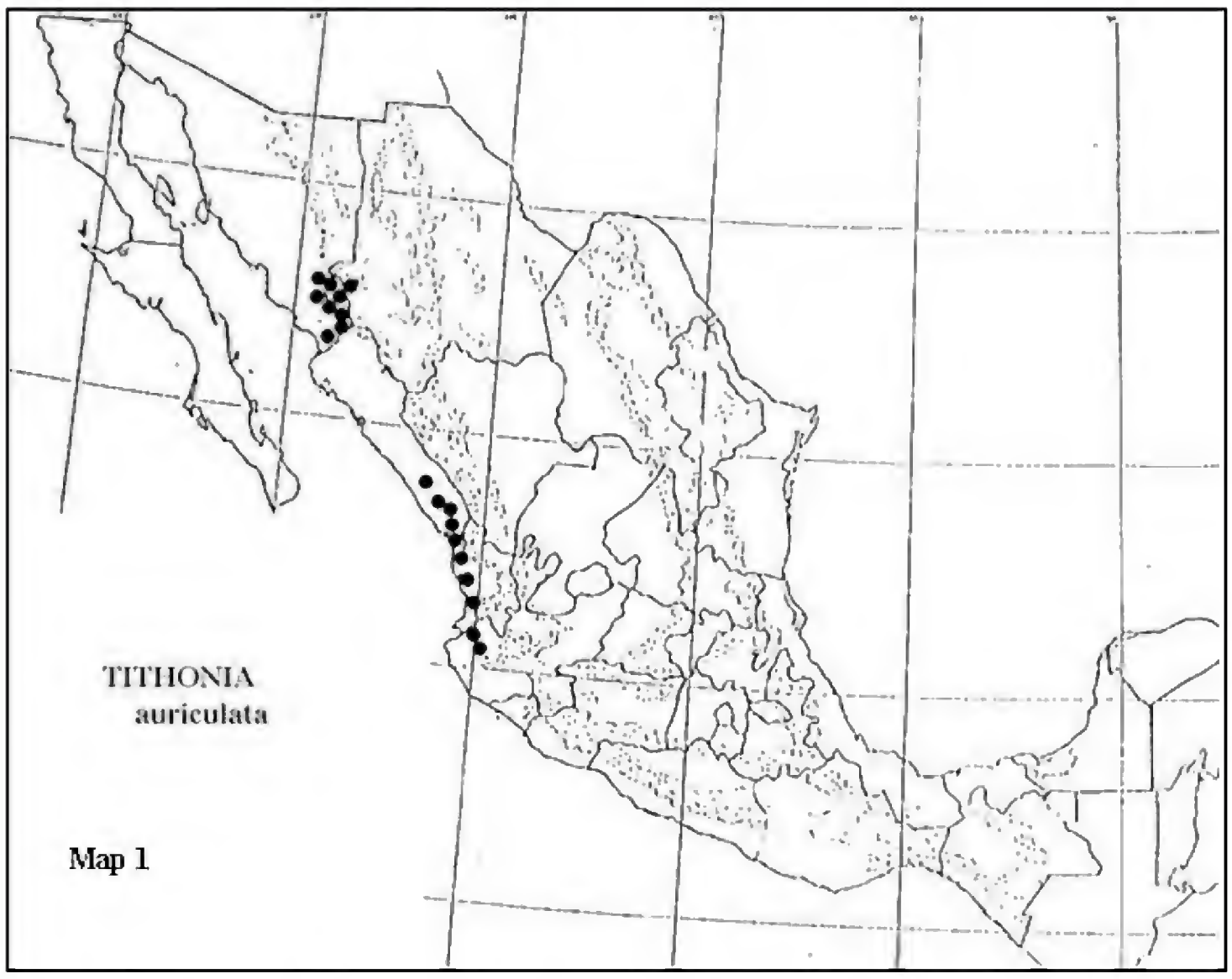
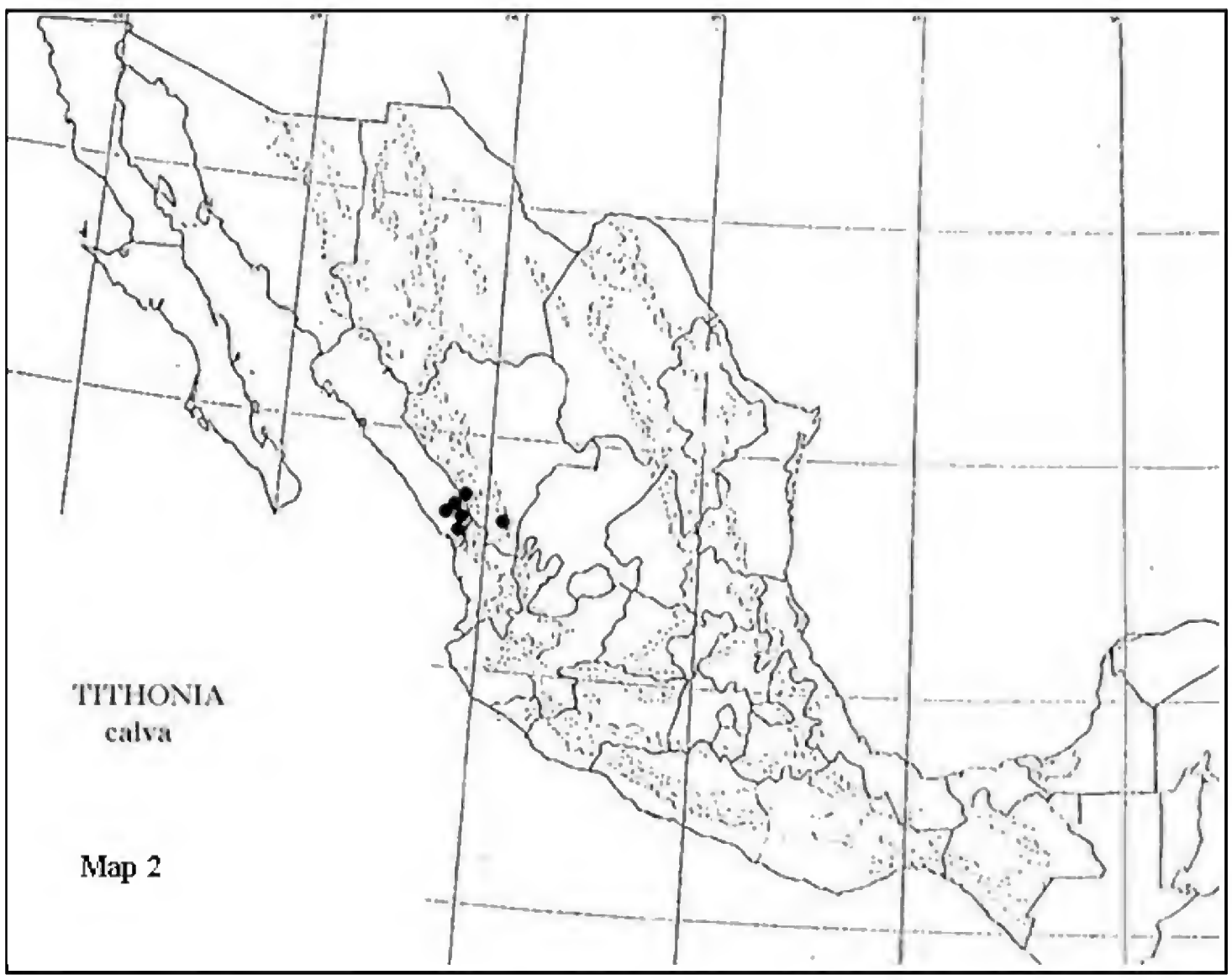


TITHONIA
auriculata

Map 1



TITHONIA
calva

Map 2

Recension of *Viguiera* (sensu stricto) (Asteraceae: Heliantheae) of Mexico

Billie L. Turner
Plant Resources Center
The University of Texas
Austin TX 78712

ABSTRACT

A taxonomic account of the genus **Viguiera** (sensu stricto) for Mexico is rendered. Two species are recognized within the complex: a very localized edaphic endemic of northern Michoacan, **V. moreliana** B.L. Turner, **sp. nov.** and a widespread, highly variable, **V. dentata** with 4 intergrading varieties, one of these newly described, var. **longibracteata** B.L. Turner, **var nov.**, a localized gypsophile from sw Coahuila. A complete synonymy is provided, along with keys to the taxa, and maps showing their distribution, all following the format of my ongoing, Comps of Mexico Turner (2014).

KEY WORDS: Asteraceae, Heliantheae, *Viguiera*, *V. dentata*, Mexico. Published on-line **www.phytologia.org** *Phytologia 97(1): 16-24 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: Asteraceae, Heliantheae, *Tithonia*, *T. auriculata*, *T. calva*, Mexico

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VIGUIERA H.B.K. [sensu stricto]

Annual or perennial herbs, shrublets, shrubs or small trees to 5 m high. Leaves opposite below, opposite or alternate above, simple, entire, dentate or shallowly lobed. Heads radiate, solitary or arranged in a branched capitulescence. Involucres 2-7 seriate, the bracts graduate or subequal, appressed or markedly reflexed. Ray florets neuter, sterile, the ligules yellow. Disk florets numerous, yellow. Achenes obovate, plump at maturity, radially compressed, the margins without wings, the pappus, when present, of 2 lateral awns between which are present 2-8 lacerate short scales. Base chromosome number, x = 17.
Type species, *Viguiera dentata* (Cav.) Spreng.

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As treated by Blake (1918), **Viguiera** was conceived as a large genus with perhaps 150 species. McVaugh (1984) and Panero (2006) essentially accepted Blake's circumscription. Schilling and Panero (2011), largely on DNA data, drastically revised **Viguiera** and related genera, restricting the latter to a single species, the widespread, commonly encountered, **V. dentata**, this readily identified by the pubescent filaments of its stamens (other taxa lacking such hairs). In addition to their earlier establishment of **Bahiopsis**, **Davilianthus**, **Heliomeris**, **Hymenostephium** (Schilling and Panero 2002), these carved out of Blake's **Viguiera**, they also recognized four newly described genera from within its midst: **Dendroviguiera**, **Gonzalezia**, **Heiseria** and **Sidneya**, at the same time expanding **Aldama** (including *Rhysolepis*) to about 120 taxa. All very difficult, but the authors did provide a partial key to the complex, intercalated with yet other genera of the tribe Heliantheae, this difficult to use, as might be expected following such extraordinary reevaluations.

Early on, I accepted a monotypic **Viguiera**, as espoused by Schilling and Panero (2011), readily recognized by its markedly pubescent filaments, the only Mexican species to possess this trait (H. Robinson, 1977). Previous workers accepted several intergrading infraspecific taxa within the **V. dentata** complex. In the present reappraisal of **Viguiera**, I treat the genus as consisting of two species: **V. dentata**, a widespread taxon having 4 intergrading varieties, and the newly proposed **V. moreliana**, confined to the state of Michoacan, and without evidence of intergradation with **V. dentata** or its varieties.

Key to species

1. Peduncles and petioles pubescent with loose spreading trichomes 1.0-1.5 mm long; petioles wingless, abruptly passing into the broadly subcordate blades; north-central Mic.....**V. moreliana**
1. Peduncles and petioles pubescent with stiff appressed hairs; petioles with a pronounced wing or not, these often passing into the blades; absent from n Mic.....**V. dentata**

VIGUIERA DENTATA (Cav.) Spreng., Syst. 3: 615. 1826.

This is an extremely variable, widespread species, forms of which may mimic this or that taxon, making it difficult to key using leaf or floral characters. Fortunately, so far as known, it is the only Mexican species of **Viguiera** with pubescent anther filaments. Nevertheless, occasional specimens of this or that taxon appear to have glabrous filaments, or nearly so (e.g., *Sharp 441041*, NY; etc.).

Blake (1918) recognized what he thought were 4, well-marked, varieties within **V. dentata.** I can recognize 3 of these, but they do not appear to be especially well-marked, since I find considerable intergradation among them. I have added a fourth varietal taxon (var. **longibracteata**) to the complex, plus a new species, **V. moreliana**, confined to north-central Michoacan, the latter having densely pubescent staminal filaments, but it does not grade into **V. dentata**.

Key to varieties

1. Outermost involucral bracts 1-3 cm long; mostly gyp soils of southwestern Coa .....var. **longibracteata**
1. Outermost involucral bracts mostly 0.5-1.0 cm long; widespread...(**2**)

2. Leaves lanciform, the petioles tapered-upon to the base or nearly so; capitulescence few-headed, the ultimate peduncles mostly 2-10 cm long; Sierra Madre Occidental, Son, Chi, Sin, Dur .....var. l**ancifolia**
2. Leaves broader, ovate to deltoid, or if lanciform then densely pubescent beneath; capitulescence more compact, few- to numerous-headed on ultimate peduncles mostly 1-6 cm long; s Central Plateau and Gulf coastal slopes, Chi to Cps ...(**3**)

3. Leaves densely and softly pubescent beneath, usually winged; heads relatively large, Central Plateau (Zac, Agu south to Cps) .....var. **canescens**
3. Leaves otherwise; heads smaller; n and e Central Plateau and Gulf slopes (Chi, Nue s to Cps, Cam, Yuc, Qui) ..... var. **dentata**

var. **dentata**
Encelia montana Brandegee
Helianthella latifolia Scheele
Helianthus dentatus Cav.
Helianthus triqueter Ort.
Viguiera brevipes DC.
Viguiera dentata var. *brevipes* (DC.) Blake
Viguiera grammatoglossa DC.
Viguiera helianthoides H.B.K.
Viguiera dentata var. *helianthoides* (H.B.K.) Blake
Viguiera laxa DC.
Viguiera laxa var. *brevipes* (DC.) A. Gray
Viguiera microcline DC.
Viguiera oppositipes DC.
Viguiera pedunculata Seaton
Viguiera sagraeana DC.
Viguiera texana Torr. & Gray

USA: Ariz, N. Mex and Tex. **MEX:** Son, Chi, Coa, Nue, Tam, Sin, Dur, Zac, San, Nay, Jal, Col, Gua, Que, Hid, Mic, Mex, Pue, Ver, Gue, nw Oax, Cps, Yuc, Cam, Qui and Guatemala southwards, weedy along roadsides and in cleared fields, mostly dry shallow soils, 1000-2000 m; all seasons. **Map 1**
Erect perennial herbs 50-150 cm high; leaves mostly alternate above, rarely opposite throughout, 5-15 cm long, 3-8 cm wide, petioles 1-5 cm long, taperingly-winged throughout or not; blades exceedingly variable as to shape, but mostly ovate to ovate-deltoid, very rarely 3-lobed, 3- or 5-nervate from, or above, the base, the margins entire to coarsely dentate, rather hispid or rough-pubescent beneath; heads radiate, few to numerous in terminal branched panicles; involucres campanulate to hemispheric, 3-4 seriate, the bracts graduate to subequal; rays 5-13, the ligules yellow, 8-20 mm long; achenes pubescent, pappose with 2 slender awns 2-3 mm long, the intervening scales mostly un-united, 0.5-1.0 mm long; chromosome number, n = 17 pairs.

This broad-ranging, highly variable, taxon grades into var. **lancifolia** in the southwestern USA and northwestern Mexico, into var. **canescens** along the western part of its range from s Zac to Cps and, more locally, into var. **longibracteata**. Occasional specimens of var. **dentata** may be found well within the range of var. **canescens** (e.g., Col), but these are believed to be recent introductions.

The inclusion of *V. grammatoglossa* as a synonym in the above is based upon Schilling and Panero (2010); I erred (Turner 2011) in treating the name within **Davilianthus** (as shown in my Map 1, but no such combination was made for the taxon).

var. **canescens** (DC.) Blake, Contr. Gray Herb. 54: 87. 1918.
Viguiera canescens DC.
Viguiera longiligula M.E. Jones
Viguiera nelsonii Rob. & Greenm.

Mostly w part of Central Plateau, n Jal, s Zac, Agu, Gua, Que, Mic, Mex, Mor, Gue, Oax, Cps and Guatemala, roadsides and old fields, 1000-2200 m; all seasons. **Map 2**

Much-resembling var. **dentata**, but the leaves lanceolate and densely soft-canescent beneath, the blades usually tapering upon the petioles as a distinct wing; chromosome number, n = 17 pairs.

This taxon grades into var. **dentata** to the north and east.

var. **lancifolia** Blake, Contr. Gray Herb. 54: 86. 1918.

Son, w Chi, n Sin, n w Dur and adjacent USA, dry hills and roadsides, 1000-2200 m; all seasons. **Map 1**

Much-resembling var. **dentata** but the leaves mostly lanceolate; chromosome number, n = 17 pairs.

The var. **lancifolia** is a weakly differentiated taxon but clearly a regionally defined gene-pool of the Sonoran Desert regions within which forms referable to either var. **dentata** and var. **cinerascens** are largely excluded; nevertheless, occasional intergrades with var. **dentata** occur, as noted in Map 1.

var. **longibracteata** B.L. Turner, **var. nov. Fig. 1**

Much-resembling var. **dentata** but readily recognized by its 10, elongate, outermost, involucral bracts, these up to up 3 cm long.

Perennial herbs or shrublets, 0.8-1.0 m high. Leaves (larger) 8-12 cm long, 3-6 cm wide; petioles 2-5 cm long; blades broadly lanceolate to broadly ovate, moderately appressed-pubescent above and beneath, 3-nervate from or near the base. Heads 1-4, arranged in terminal cymes, the peduncles 2-10 cm long, pubescent with upwardly appressed hairs. Involucres 2-4 seriate, imbricate, the outermost series of ca 10 lanceolate bracts, 1-3 cm long. Pales rigid, 5-7 mm long, their apices 2-4 mm long, indurate, yellow. Ray florets 8, sterile; ligules yellow, 8-12 mm long. Disc florets numerous, yellow, 4.5-6.0 mm long; corollas ca 5 mm long. Anthers brown; filaments sparsely pubescent. Achenes 3-4 mm long, the two lateral awns deciduous, between these, 2-4 persistent, membranous scales ca 0.5 mm high.

TYPE: **MEXICO. COAHUILA:** "in gypsum outcropping, on north side of Sierra de los Organos, about 5 air milles SW of Cuesto de Gallo," 4400 ft, 26 44 N, 103 03 W, 8 Aug 1973, *J. Henrickson 12111* (Holotype: LL-TEX). **Map 1**

ADDITIONAL SPECIMENS EXAMINED: **MEXICO: COAHUILA:** ca 10 mi W of Ejido Providencia, 19 Oct 1971, *Bacon 1240* (TEX); Sierra Mojada, just S of Esmeralda, 1 Sep 1972, *Chiang et al. 9081* (LL-TEX); 1.5 mi SW of Las Delicias, 3900 ft, gypsum outcrops, 15 Aug 1973, *Henrickson 12453a* (LL-TEX); 1 mi SW of Las Delicias, 3560 ft, 27 Aug 1971, *Henrickson 6048* (LL-TEX); 1.5 mi SW of Las Delicias, 3600 ft, in gypsum, 12 Aug 1973, *Henrickson 12274* (LL-TEX); E of Cinco De Mayo "gypsum ravine," 22 Sep 2006, *Hinton et al. 28471* (TEX); N of Laguna del Rey, 1094 m, 26 Sep 2009, *Hinton et al. 28848* (TEX); SW end of Sierra de la Fragua, "near crest, one colony," 2 Sep 1941, *I.M. Johnston*

8752 (LL-TEX); 9.5 mi E of Puerto del Gallo in "pure gypsum," 8 Aug 1973, *M.C. Johnston et al. 12138a* (LL-TEX); ca 1 km W of Las Delicias, gyp soils, 24 Mar 1973, *M.C. Johnston et al. 10389c* (LL-TEX);

As indicated in the above key, var. **longibracteata** is readily distinguished by its outermost, elongate, involucral bracts. Considering its geographic restriction and proclivity to gypseous soils, the populations concerned seem worthy of varietal rank. It appears to grade into populations of var. **dentata** along its perimeters, especially in northern Zac.

VIGUIERA MORELIANA B.L. Turner, **sp. nov. Fig. 2**

Perennial herbs or shrublets 1-2 m high. Leaves (larger) 6-18 cm long, 4-9 cm wide; petioles 3-4 cm long, unwunged, pubescent with mostly spreading hairs, 1.0-1.5 mm long, not at all upwardly appressed; blades broadly ovate to deltoid or subcordate, sparsely pubescent above, pubescent below with mostly spreading trichomes mainly along the venation, 3-nervate from or near the base, the margins serrate. Heads terminal, arranged 1-8 in terminal cymes, the ultimate peduncles 2-10 cm long, pubescent like the petioles. Involucres 2-4 seriate, scarcely imbricate, 8-10 mm high. Pales 8-9 mm long, purplish, their apices apiculate for ca 1 mm. Ray florets ca 15; ligules yellow, 10-20 mm long, 5-10 mm wide. Disc florets numerous; corollas yellow, ca 4.5 mm long; throat ca 0.5 mm long; lobes ca 1 mm long. Stamens brown, the filaments markedly pilose. Achenes ca 3 mm long, appressed-pubescent; pappus of two lateral awns, 2-3 mm long, between these 2-4 membranous scales ca I mm long.

This novelty is readily separated from **V. dentata** by the characters given in the above key, and by yet other features such as the short, flattened, apices of its pales (vs cylindrical and elongate), and sub-cordate leaf blades, the petioles lacking wings.

TYPE: **MEXICO. MICHOACAN: Mpio. de Morelia,** Cerrito Estiladero, al N de Buenavista, 2580 m, 17 Nov 1998, *Sergio Zamudio R. 10917* (Holotype: TEX)

According to the collector, the plant was a perennial herb ca 2 m tall and "abundante" in oak forests growing among igneous rocks. **Map 3**

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. MICHOACAN: Mpio. de Morelia,** "Cinco Cerritos cerca de Tancitaro," 2100 m, 8 Jul 1992, *Escobedo Garcia 2307* (TEX); **Mpio. la Piedad**, "Parte mas alta del Cerro Grande de Cujarurato, al SW de la Piedad," 2500 m, 16 nov 1971, *Rzedowski & Mcvaugh 526* (TEX). **Mpio. Zacapu,** "2 km de Zacapu, sobre la carretara a Zamora...encinar perturbado sobre corriente de lava basaltica," 2100 m, 23 Oct 1987, *Diaz Barriga 4545* (TEX).

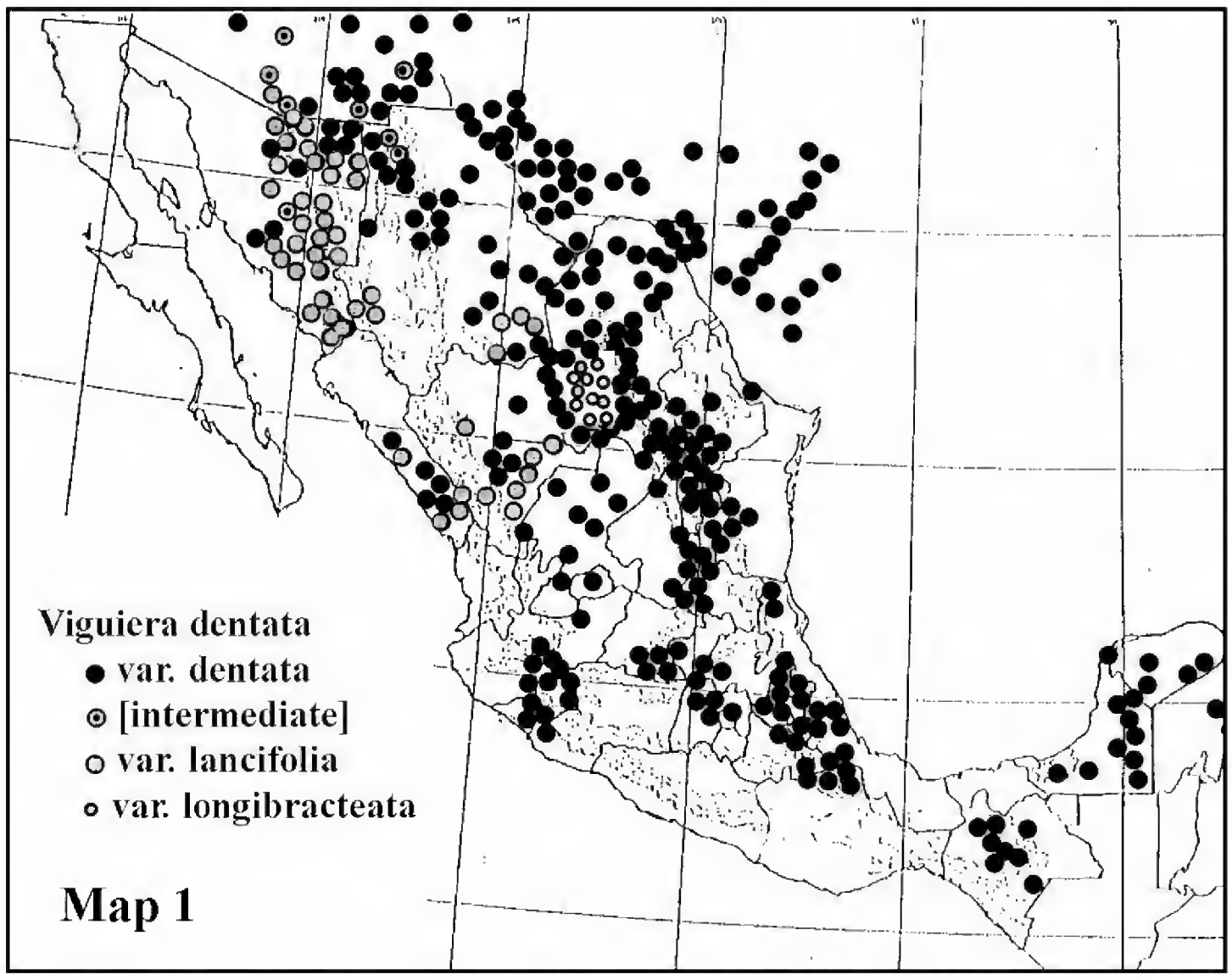
To judge from label data, the species occurs in oak forests among lava rocks in the vicinty of recent and ancient volcanic activity.

The several cited specimens are remarkable uniform and are readily distinguished from all of the named verities of **V. dentata**; Blake (1918) does not cite specimens of the latter from the state of Michoacan, nor have yet other workers. I consider the taxon to be an edaphic endemic, confined to lava outcrops in the region concerned.

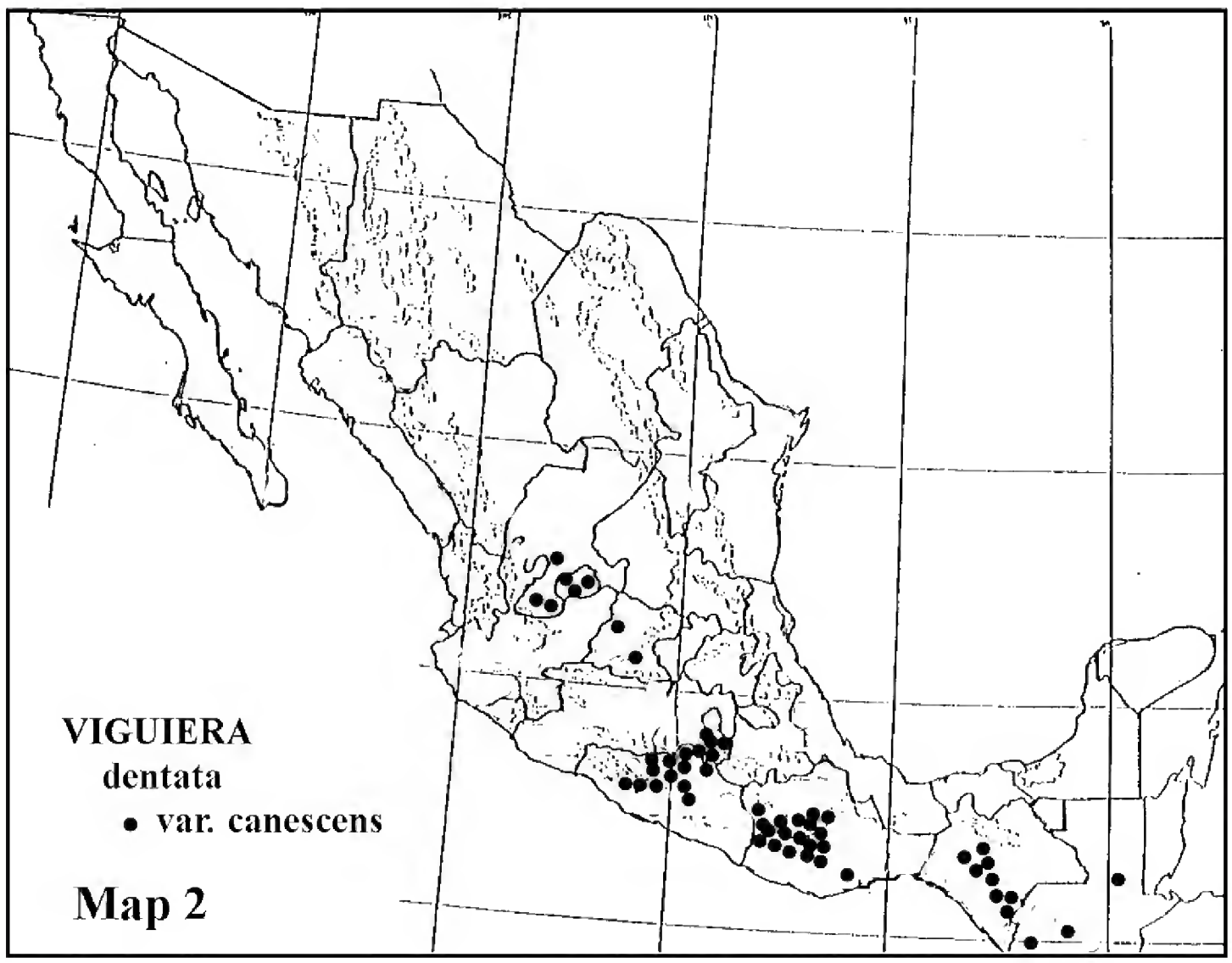
The species is named for the Municipio de Morelia, whence the type.

ACKNOWLEDGEMENTS

I am grateful to P.E. Berry of MICH for providing meaningful specimen data from their collections, and to Jana Kos for proofreading the paper.


Viguiera dentata
● var. dentata
◉ [intermediate]
○ var. lancifolia
○ var. longibracteata

Map 1


VIGUIERA
dentata
• var. canescens

Map 2

Figure 1. *Viguiera dentata* var. *longibracteata*.

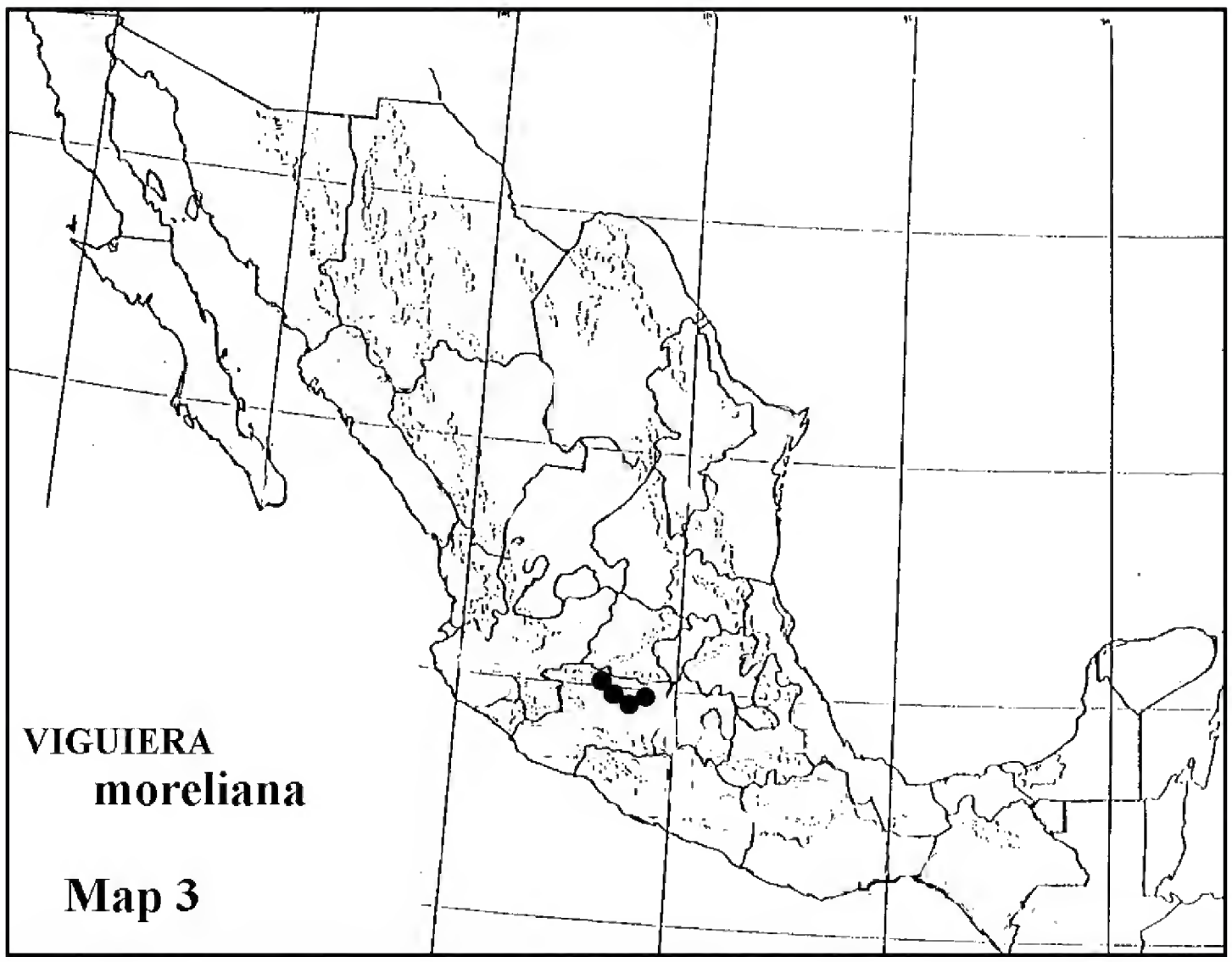

VIGUIERA
moreliana
Map 3

Figure 2. *Viguiera moreliana.*

Three new species of *Stevia* (Asteraceae: Eupatorieae) from northern Mexico

Billie L. Turner, Plant Resources Center,
The University of Texas, Austin TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

Three new taxa of **Stevia** are described: two from northwestern Mexico, **S. puricana** B.L. Turner, **sp. nov.** and **S. porphyreoides** Yahara & Soejima, **sp. nov**. and **S. zaragozana** B.L. Turner, **sp. nov.** from southern Nuevo Leon. The former two taxa relate to *S. porphyria*, the latter to the recently described *S. viejoana* Soejima et al. Photographs of the types and distribution maps are provided. Published on-line **www.phytologia.org** *Phytologia 97(1): 25-31 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: Asteraceae, Eupatorieae, *Stevia, S. porphyria, S. viejoana*, Mexico, Sonora, Chihuahua, Nuevo Leon

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Stevia is a relatively uniform large genus of the more tropical regions of the Americas with 100 or more species occurring in Mexico, many of these localized endemics (Grashoff 1972; Soejima, Yahara and Watanabe 2001; Watanabe, Yahara, Soejima and Itos 2001; Turner 1997; Turner 2013a; Turner 2013b). The present account adds an additional three species to the list.

STEVIA PURICANA B.L. Turner, **sp. nov. Fig. 1.**

Stiffly erect, perennial herbs, 50-70 cm high, mostly unbranched. **Stems** pubescent with crinkly hairs 0.5-1.0 mm high. **Leaves** alternate, 2-5 cm long, 0.3-1.0 cm wide; petioles obscure, passing into the blades; blades linear-obovate to ovate, sparsely pubescent and glandular punctate on both surfaces, the margins weakly dentate. **Capitulescence** a terminal, corymbose-panicle, 4-8 cm across, 4-6 cm high, the ultimate peduncles 1-6 mm long. **Heads** mostly 8-10 mm long; involucral bracts linear-lanceolate, 5-6 mm long, sparsely pubescent with acute apices. **Corollas** mostly purple, 5-6 mm long; tubes passing into the throats; lobes ovate, pubescent, white beneath, ca 1.5 mm long. **Achenes** black, ca 4 mm long, sparsely pubescent apically; adelphocarps with 2-3 stout, yellowish, slender, apically ciliate, scales 3-4 mm long, between these 1-2 membranous scales ca 1 mm long; idiocarps with membranous scales ca 1 mm long.

TYPE: **MEXICO. SONORA: Mpio. de Nacozari de Garcia,** "Antennas on top of Sierra la Purica, 19.1 km (by air) NNW of Nacozari, Reserva Forestal Nacional y Refugio de Fauna Silvestre Ajos-Bavispe, 2467 m, 30 32 39 N, 108 54 55 W, pine forests, 9 Sep 2013, *A.L. Reina-G.* et al *2013-320* (Holotype: TEX). **Map 1**

ADDITIONAL SPECIMEN EXAMINED: Same locality and date: *Reina-G.* et al *2013-408* (TEX).

Named for the Sierra Purica, Sonora, whence the type. According to Google, the upper portions of the Sierra Purica are dominated by *Pinus arizonica* and *P. engelmannii*.

In my treatment of **Stevia** for Mexico, this novelty will key to or near **S. porphyria** McVaugh, a widespread variable species of central Mexico. It differs from the latter in having smaller heads, smaller involucres and achenes with stout, sclerotic, pappus scales; it might also be compared with the newly proposed **S. porphyreoides**, the latter possessing similar sized heads, but readily recognized by its slender pappus bristles.

The following key will choose among them:

1. Heads mostly 10-12 mm long; phyllaries mostly 6.5-8.0 mm long; corolla lobes oblong–elliptic; central and north-central Mexico .....**S. porphyria**
1. Heads mostly 8-10 mm long; phyllaries mostly 5-7 mm long; corolla lobes linear to narrowly ovate; northwestern Mexico...(**2**)

2. Achenes bearing capillary bristles; corolla lobes more nearly linear; membranous scales ca 0.6 mm long; Chi, Dur and n Jal.....**S. porphyreoides**
2. Achenes bearing sclerotic awns or scales, bristles absent; membranous scales ca 1 mm long; corolla lobes ovate; Sierra Purica, ne Sonora.....**S. puricana**

STEVIA PORPHYREOIDES Yahara and Soejima, **sp. nov. Fig. 2**

Stiffly erect rhizomatous perennial herbs, mostly unbranched, 50-80 cm high. **Stems** pubescent with crinkly hairs, 0.5-1.0 mm high. **Leaves** mostly alternate, sessile, 2.0-3.5 cm long, 0.4-0.8 mm wide; glandular punctate on both surfaces, weakly crenate. **Capitulescence** a terminal corymbose panicle up to 8 cm across, the ultimate peduncles 1-6 mm long. **Heads** 8-9 mm long; involucres 5-7 mm long, the apices acute. **Corollas** deep purple, sparsely pubescent, tube plus throat 4-5 mm long; lobes linear, 1.0-1.2 mm long. **Achenes** heteromorphic, ca 3 mm long, aristate, sparsely hispid; pappus of adelphocarps having 2-3 slender awns, 5-6 mm long, the membranous scales ca 0.6 mm long; that of the idiocarp a crown of united scales ca 0.6 mm high.

TYPE: **MEXICO. DURANGO: Mpio. de El Mezquital,** "22 Km al NE de Los Charcos." 2750 m, 1 Nov 1982, pine-oak woodlands, *S. Gonzalez & J. Rzedowski 2356* (Holotype: TEX). **Map 1**

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. CHIHUAHUA**: near Colonia Garcia, 7500 ft, 7 Sep 1899, *Townsend & Barber 310* (TEX). **DURANGO:** "Rancho La Pena (el Bebedero), Suchil." Oak forests, 7 Oct 1882, *Gonzalez E. 1110* (TEX). **JALISCO:** "ca 8-10 km SE of El Mortero, near Mezquitic, on Zacatecas-Jalisco border, along the road to Monte Escobedo, Zacatecas," 2450 m, 5 Nov 1963, *Feddema 2457* (TEX).

McVaugh (1984) did not cite the *Feddema* collection noted above, but Grashoff annotated a duplicate sheet (TEX) as "*S. porphyrea* JCG 1972." Indeed, McVaugh, in his Flora Nova-Galiciana acknowledges but a single collection of the latter from Jalisco (SW of Ojuelos; *McVaugh 16582*), this close to the area from whence the Type (Aguacalientes), as indicated in Map 1.

Yahara and Soejima sent me an advanced copy of their manuscript, "Two new species of *Stevia* from Mexico," for review. This I rendered, but the authors chose not to describe the novelty concerned, perhaps because I questioned its validity, largely based upon the Grashoff collection cited above, Grashoff being my Academic Son, this creating a bias.

Description of my proposed **Stevia puricana** has made me reexamine the **S. porphyria** complex, based upon specimens on file at TEX, and I conclude that **S. porphyreoides**, as proposed by Yahara and Soejima, has nomenclatural merit, although, morphologically, very close to **S. porphyria.** According to its authors, **S. porphyreoides** produces "abundant" pollen grains and is "considered to be sexual [as opposed to apomictic]."

It should also be noted that the joint authors questioned the *Townsend & Barber* collection from nw Chi, with the observation that it differs from typical **S. porphyreoides** in having "4 adelphocarps of 1 awn, a higher crown of united scales of the idiocarp (ca. 0.9 mm high) and densely sessile-glandular florets." I have included it herein largely on its overall gestalt, but it could possibly prove to be novel, considering the characters concerned, and its geographic isolation from the more southern populations (cf Map 1).

STEVIA ZARAGOZANA B.L. Turner, **sp. nov. Fig. 3.**

Perennial herbs to 25 cm high. **Mid-stems** markedly pubescent with crinkly hairs 0.5-1.0 high. **Leaves** (mid-stem) 2-3 cm long, 1-2 cm wide; petioles 2-4 mm long, passing into the blades; blades ovate, widest near the middle, sparsely pubescent above and below, 3-nervate from or near the base, the margins serrulate. **Capitulescence** a terminal array of congested heads, collectively 6-7 cm across, ca 10 cm high; heads 0.5-2.0 cm across and as high, the ultimate peduncles sessile, or nearly so. **Involucres** mostly 8-9 mm long, glabrous, or nearly so, their apices sharply acute. **Corollas,** purple, glabrous or nearly so; tube ca 1.5 mm long, passing into the throat; lobes ovate, ca 1.5 mm long. **Achenes** ca 4 mm long, black, wingless, glabrous; pappus a crown of lacerate scales ca 0.5 mm high.

TYPE: **MEXICO. NUEVO LEON: Mpio. Zaragozana,** "Canada La Tinaja, between Rancho La Encantada and Cerro La Pena." 2600-2700 m, 23 56 N, 99 49 W, 3 Jul 1988, "Pine-oak-madrono association on northern exposure." *T. F. Patterson 5787* (Holotype: TEX). **Map 2**

In my treatment of **Stevia** for Mexico (Turner 1997), this novelty will key directly to **S. hintoniorum** B.L. Turner; it is readily distinguished from that taxon by a number of characters, including habit, leaf shape, etc. It might also be compared with the recently described **S. viejoana** (Soejima et al 2001), this also occurring in Mpio. Zaragozana (Map 2), the latter differing in having lanceolate to oblong-lanceolate leaves, the upper stems stipitate-glandular, involucres smaller (ca 5 mm high vs 8-9 mm) and glabrous achenes.

Named for the Mpio. Zaragozana, whence the Type.

ACKNOWLEDGEMENTS

Thanks to Yahara and Soejima for providing useful information about their **S. porphyreoides.** Jana Kos proofed the paper, providing helpful comments.

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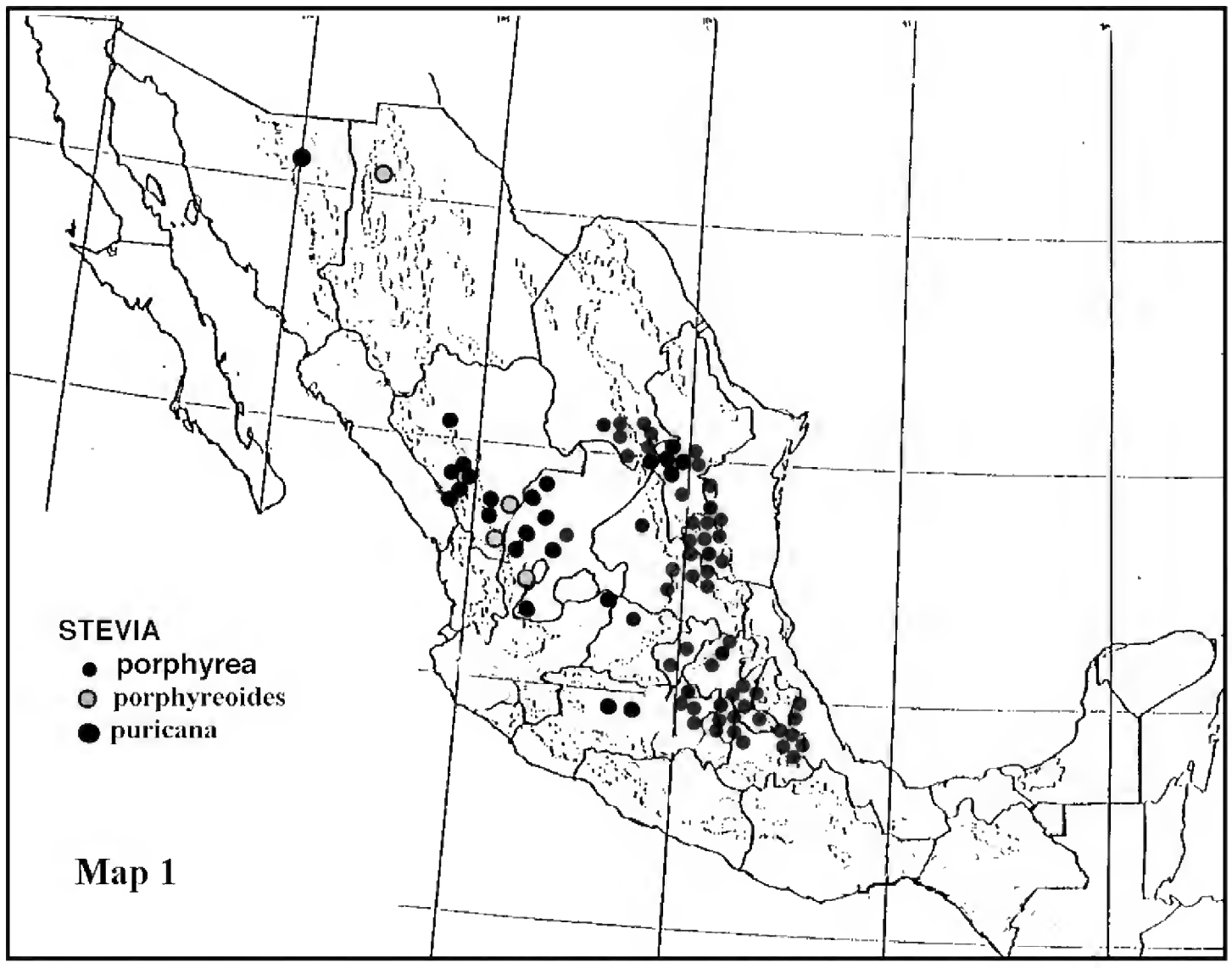
STEVIA
porphyrea
porphyreoides
puricana

Map 1


STEVIA
viejoana
zaragozana

Map 2

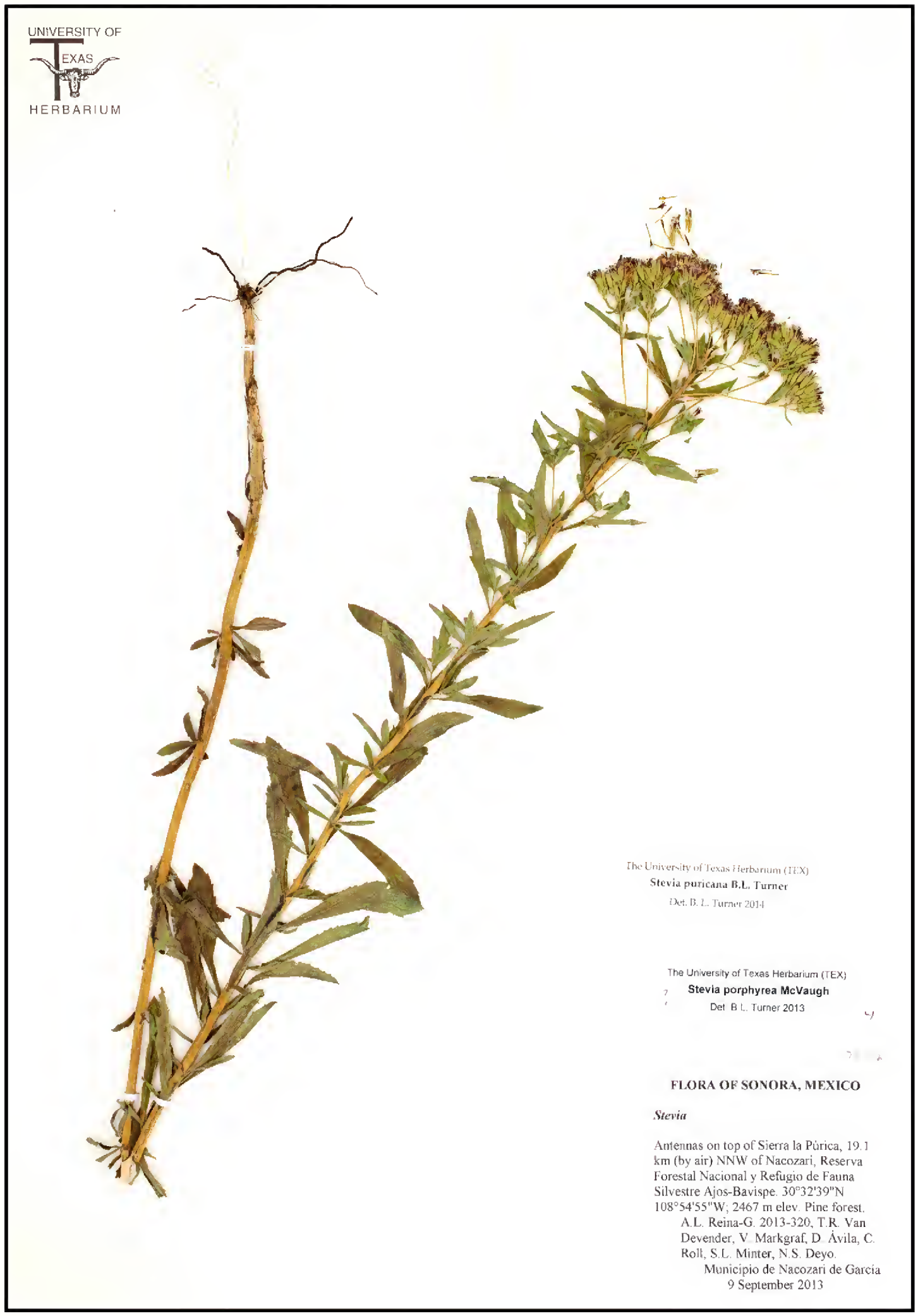

Figure 1. *Stevia puricana.*

Figure 2. *Stevia porphyreoides.*

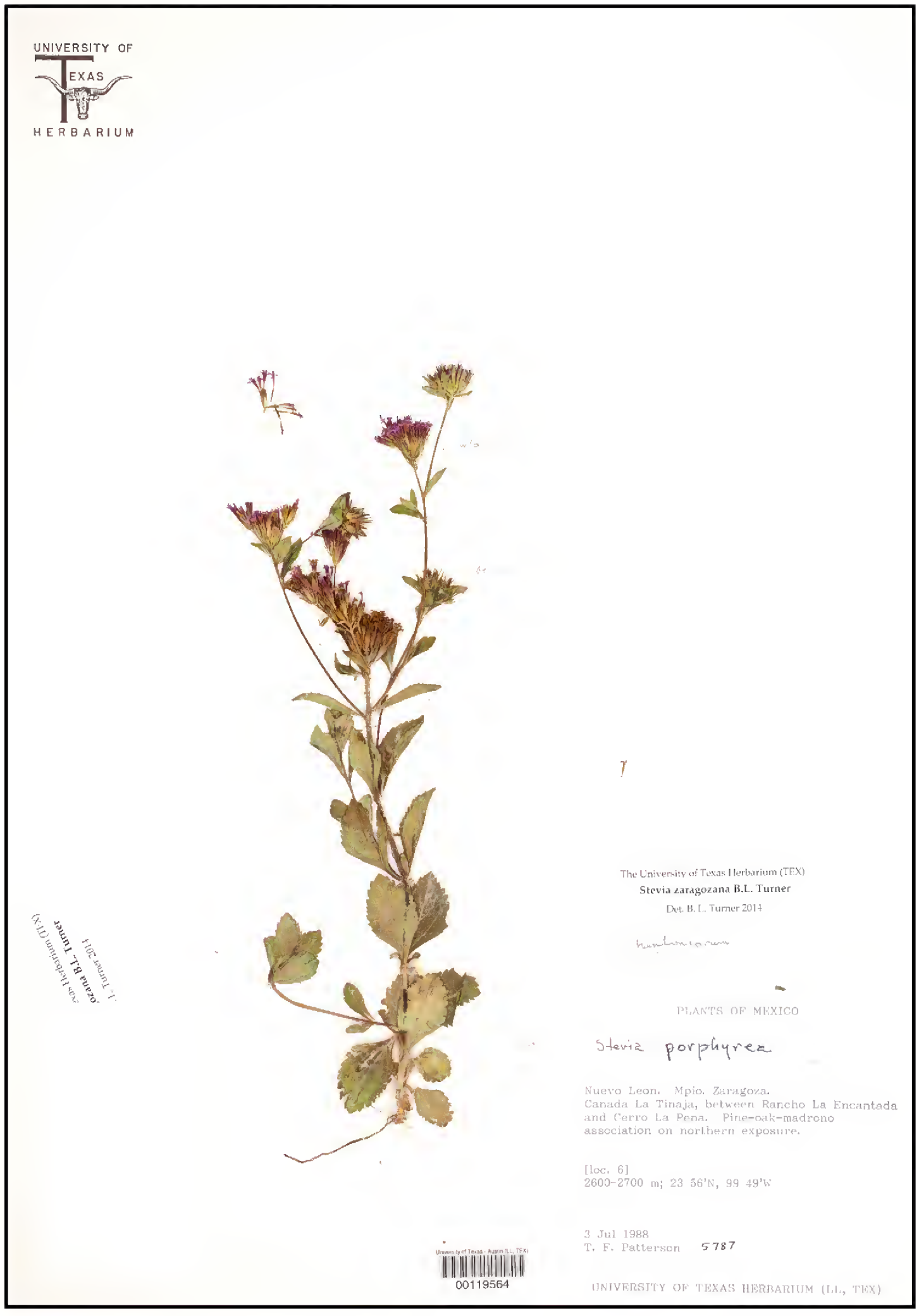

Figure 3. *Stevia zaragozana.*

Gas exchange rates for *Chaptalia texana* (silver-puff, Asteraceae) An herbaceous understory species

O. W. Van Auken
Department of Biology, University of Texas at San Antonio
San Antonio, Texas 78249, correspondence to oscar.vanauken@utsa.edu

and

J. K. Bush
Department of Environmental Science, University of Texas at San Antonio
San Antonio, Texas 78249

ABSTRACT

Chaptalia texana Greene (silver-puff) is found in parts of southwestern North America including central and western Texas, southern New Mexico, and Mexico. We examined the local density distribution of *C. texana* to determine its habitat preference. We found *C. texana* below the canopy of *Juniperus-Quercus* (juniper-oak) mottes or woodlands, and not in adjacent C_4 grasslands. *Chaptalia texana* density was highly variable with 0-15 plants/m^2 below the woodland canopy and none in adjacent C_4 grassland. We examined its gas exchange rates to see if it was a habitat specialist. Maximum gas exchange rates (A_{max} ± SD) of leaves of *C. texana* growing below the canopy were 19.24 ± 1.23 μmol·CO_2/m^2/s at mid-day open area light levels (photosynthetically flux density [PFD] = 2098 ± 41 μmol/m2/s). This was 3.91 times higher than its sub-canopy mid-day ambient CO_2 uptake rate at sub-canopy mid-day light levels (PFD = 545 ± 86 μmol/m^2/s). Rates of other measured physiological characteristics of *C. texana* plants in shade as compared to rates of known shade plants were high. Mean light intensities (10:00-17:00 hr) below the canopy were approximately 20% of the full sun light levels, and were 1.46 times higher than *C. texana* light saturation. Gas exchange rates for this species at sub-canopy light levels were similar to other understory or shade species, while gas exchange rates for this species at open gap or grassland light levels were similar to other open grassland or sun species. This species seems best described as photosynthetically facultative; however results do not explain why *C. texana* plants were not found in the open grasslands. Published on-line **www.phytologia.org** *Phytologia 97(1): 32-44 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: Photosynthesis, CO_2 uptake, woodlands, savannas, shade leaves, light levels, understory, canopy, *Chaptalia,* silver-puff.

Many arid and semi-arid grassland communities have undergone encroachment by woody plants and are now some type of woodland or savanna (Begon et al. 2006; Reynolds et al. 2007; Bond 2008; Van Auken 2009). Savannas have woodland and grassland phases with plant species usually restricted to one phase or the other, but reasons for the restrictions are not clear. Transformations of grasslands have taken place throughout the world (Van Auken and McKinley 2008; Maze 2009) involving encroachment or invasion of a few woody species with as many as 10% of the herbaceous grassland species being true invaders (Van Auken 2009). Included are many of the woodland and grassland communities of the central Texas Edwards Plateau which vary from east to west along a rainfall gradient (more arid in the west, Van Auken and McKinley 2008; Van Auken and Smeins 2008).

Chaptalia texana Greene (silver-puff, Asteraceae) is an herbaceous, perennial, rosette plant reported from the woodland phase of some central Texas savanna communities (Correll and Johnston 1979; Enquist 1987; USDA 2009; Harms 2011). It seems to be an understory species found below the canopy of some of these woodlands (personal observation) but reasons for its occurrence below the

canopy are undetermined. Physiological differences between plants native to open, full-sun habitats as compared to those found in shady, understory communities are fairly well known (Begon et al. 2006; Valladares and Niinemets 2008; Smith and Smith 2012). Typical shade species have low maximum photosynthetic rates (A_{max} values < 7.0 μmol·CO_2/m^2/s), low light saturation, light compensation, dark respiration rates, conductance, and transpiration compared to sun adapted species that have A_{max} values > 15.0 μmol·CO_2/m^2/s and other physiological characteristics that are high (Begon et al. 2006; Valladares and Niinemets 2008; Smith and Smith 2012). In addition, most if not all understory or shade plants are C_3 plants, many with one layer of horizontal leaves, large leaf surface area, thinner leaf blades, and more chlorophyll per mass but less per unit area (Begon et al. 2006; Valladares and Niinemets 2008; Smith and Smith 2012). *Chaptalia texana* has some of these morphological characteristics including one layer of horizontal leaves and large leaf blades (personal observation).

No reports have been found that consider gas exchange rates for any species of *Chaptalia*. No ecological studies have been identified that might assist in ascertaining why *Chaptalia* species are found in shaded canopy environments. In addition the successional status, disturbance requirements, densities, or resource requirements of any species of *Chaptalia* are not known. However, one study of the southeastern coastal plain longleaf and slash pine forests in Georgia examined successional herbaceous species changes after fire and reported the presence of *C. tomentosa* after prescribed winter fires with cover reductions by about half eight years after the fire (Lemon 1949). In addition, there are some reports of related *Chaptalia* species in grazed grasslands in South America, suggesting that frequent disturbances such as fire, clipping or herbivory of adjacent species promoted *Chaptalia* density (Fidelis et al. 2010; Joiner et al. 2011).

Gas exchange rates of some central Texas savanna community species have been examined. One true understory species (*Carex planostachys*) had low gas exchange rates (Wayne and Van Auken 2009). A series of C_4 grasses (sun species) from open habitats in this same area had high gas exchange rates at high light levels and much lower rates at low light levels, apparently preventing them from occurring below the canopy (Wayne and Van Auken 2012). *Sophora secundiflora* (leguminosae, Texas mountain laurel, a shrub), four asteraceae (one herbaceous perennial Verbesina *virginica* and three sub-shrubs *Simsia calva, Wedelia texana, and Brickellia cylindracea*), and a Malvaceae sub-shrub (*Malvaviscus arboreus* var. *drummondii*) all had intermediate levels of gas exchange and were considered facultative species and all were found below the woodland canopy, at the canopy edge and sometimes in open areas (Furuya and Van Auken 2009; Gagliardi and Van Auken 2009; Furuya and Van Auken 2010; Van Auken and Bush 2011).

The purposes of this study were to measure the density of *C. texana* below the canopy of *Juniperus ashei-Quercus virginiana* var. *fusiformis* woodlands and adjacent open savanna grasslands. In addition, we measured ambient light levels in the open and below the canopy on clear and cloudy days. Gas exchange rates of leaves at ambient light levels and light response curves of *C. texana* plants growing below the canopy were also measured. Based on the habitat where *C. texana* is reported to occur, we hypothesized that it was a shade species. We also hypothesized that it would have low gas exchange rates including low maximum photosynthetic rates, low light saturation, light compensation, and low dark respiration, conductance, and transpiration compared to sun adapted species.

Study Species

Approximately 60 species of *Chaptalia* are found in the southern United States, Mexico, the Caribbean, and South America (Flora of North America 2003). Our study species, *Chaptalia texana,* is reported from south-western Texas, south-central New Mexico, and most of Mexico. It is a native, herbaceous, perennial that grows as a rosette of basal leaves with scapose, monocephalous stems (single flowering head or inflorescence) (Nesom 1995). Consequently, it is similar to other species of *Chaptalia.* The flowering scapes can be 13-34 cm tall at anthesis and become longer in fruit. Leaves are obovate to

ovate or elliptical and relatively thick with dense, gray-white pubescence below and dark green and glabrescent above (Enquist 1987; Nesom 1995). It is reported from thin, rocky, limestone soils and mostly from oak, pine-oak and juniper-oak woodlands (Enquist 1987; Nesom 1995; Harms 2011). It potentially flowers year round, but mainly when temperatures and rainfall are moderate. Achenes (one seeded fruits) start to germinate soon after maturation with 100% germination at 25°C in low light 16 days after the start of incubation, with slight innate seed dormancy (Van Auken 2013).

Chaptalia texana and *C. nutans* L. have sometimes been considered conspecific (Correll and Johnston 1979; Nesom 1995). Recently, it was suggested that there were two species of *Chaptalia* in central Texas including *C. texana* and *C. carduacea* Green (Harms 2011). Ranges of these species overlap in central Texas. They appear to be ecologically separated with *C. texana* being found more inland, northern and usually higher in elevation. *Chaptalia carduacea* appears to have morphological, phenological and behavioral differences from *C. texana*. Another species, *C. tomentosa* Vent., is found in east Texas, east to Florida and then north into North Carolina usually on sandy soils.

Study Site

The field site for the current study was on the southern edge of the Edwards Plateau just south of the Balcones Escarpment in northern Bexar County, central Texas. This area is rough, well-drained, with elevations increasing abruptly from approximately 200 m above mean sea level (AMSL) to 500 to 700 m AMSL. The area was a *Juniperus-Quercus* savanna with mottes (clumps) of *Juniperus, Quercus,* and other woody species with gaps or patches of grasses and other herbaceous species (Correll and Johnston 1979; Van Auken and McKinley 2008; Furuya and Van Auken 2009). All *C. texana* plants in the present field study were found in this area (approximately 29°68' N and 98°50' W).

Most of the subsurface of the study area is Cretaceous limestone, and soils are usually shallow on slopes and deep in broad valleys and flats (Taylor et al. 1962; NRCS 2006). Soils are dark colored, calcareous with usually neutral or slightly basic pH. They are clayey-skeletal, smectitic, thermic lithic calciustolls, mostly Crawford Series that are stony clay in texture, shallow to moderately deep over hard limestone with a zero to 3% slope (NRCS 2006). The soils have a non-calcareous clay surface layer which is 20-22 cm thick and a calcareous subsurface which is approximately 66 cm thick (Taylor et al. 1962).

Area climate has a mean annual temperature of 20°C with monthly means ranging from 9.6°C in January to 29.4°C in July (NOAA 2004). Mean annual precipitation is 78.7 cm, highly variable, bimodal, with peaks occurring in May and September (10.7 cm and 8.7 cm, respectively), with little summer rainfall and high evaporation. Light levels are higher in gaps or patches compared to below the woodland canopy, with seasonal significant differences in surface soil temperature and soil moisture (Wayne and Van Auken 2004; Van Auken and Bush 2011).

Area Vegetation

Juniperus-Quercus savanna was present throughout the area and is representative of savanna and woodlands in this region (Van Auken and McKinley 2008). Over all, dominant woody species in the study area were *J. ashei* (Ashe juniper) and *Q. virginiana* var. *fusiformis* (plateau live oak) with various subdominants including *Diospyros texana* (Texas persimmon), *Sophora secundiflora* (Texas mountain laurel), *Berberis trifoliata* (agarita), *Rhus virens* (evergreen sumac) and others. The major herbaceous species below the canopy was *Carex planostachys* (cedar sedge)(Wayne and Van Auken 2008), but *Malvaviscus arboreus* (wax mallow or Turk's cap) and *Pinaropappus roseus* (white rock lettuce) were also present (Van Auken and Bush 2011).

Gaps or sparsely vegetated intercanopy patches are interconnected within these woodland communities (Van Auken 2000) and various C_4 grasses and a mixture of herbaceous annuals were common. In the gaps one can find the grasses *Aristida longiseta* (red three-awn), *Bouteloua curtipendula*

(side-oats grama), *Bothriochloa laguroides* ssp. *torreyana* (silver bluestem), and *B. ischaemum* var. *songarica* (King Ranch bluestem). Some of the major herbaceous annuals included *Chaetopappa bellidifolia* (dwarf white aster), *Evax prolifera* (rabbit-tobacco), *Croton monanthogynus* (prairie-tea), *Spermolepis inermis* (scale weed), *Centaurium texense* (Lady Bird's centaury), and *Galium virgatum* (southwest bedstraw) (Van Auken 2000).

METHODS

Chaptalia Density

Four *C. texana* sub-canopy populations were identified associated with or adjacent to the grassland phase of the savanna. The density of *C. texana* within each population was counted per quadrat (m^2). Mean density of *C. texana* for each savanna community type (grassland and woodland) was calculated. We report the area of each population (canopy area), the number of *C. texana* plants counted and the density (plants/m^2) of each population.

Transects were used and consisted of 20 contiguous 1 m^2 quadrats. Measurements in the adjacent grassland phase were parallel to the *C. texana* population below the canopy and were approximately five meters from the canopy edge. The number of transects in each community type was 6 to 16 and dependent on the size of the population sampled. Seven hundred twenty total quadrats were sampled within the woodland and the same number in the grassland.

Light Levels

Photosynthetic flux density (PFD) was measured below the woodland canopy and in the open grassland. We measured PFD below the canopy in the approximate center of one population of *C. texana* and in the adjacent grassland. We used Spectrum Watchdogs (Spectrum Technologies, Plainfield, Illinois) to measure the PFD every 30 minutes over 24 hour periods from 20 April - 30 April 2012. We placed three sensors in an open grassland phase and three under the canopy. We calculated the mean PFD at each 30 minute interval using each replication (3) over 11 days. However, there were six days with full sun and three cloudy, springtime days that were grouped and are reported separately. In addition to the 24 hour light cycle, we present daily mean light levels over all daylight hours and mean midday light levels for 9 hours, 3.5 hours, and 2 hours for each community type.

Gas Exchange Measurements

Light response curves--CO_2 uptake was measured and then plotted as a function of PFD or light level for leaves of *C. texana*. Gas exchange parameters were measured on 22 April 2012 from fully expanded, basal, rosette leaves from what appeared to be mature *C. texana* plants. Leaves from five replications (individuals) were measured. Because variance was small, increasing the sample size would not change the mean values (Mendenhall and Beaver 1994). Plants that were used for making measurements were randomly selected. The average PFD outside of the chamber during the gas exchange measurements for *C. texana* was 243 ± 42 µmol/m^2/s and the soil was at field capacity. Measurements were made on leaves within ± 3 hours of solar noon with a LI-COR® 6400 infrared gas analyzer (LI-COR Environmental, Lincoln, Nebraska). The irradiances used were generated by the LI-COR® LED red-blue light source using the auto light curve program. The CO_2 concentration was 400 µmol/mol and the flow rate was 400 µmol/s. The LI-COR® 6400 was operated at approximately ambient temperature (25°C), relative humidity (35 – 57%), and was calibrated prior to making measurements. Response measurements were recorded after two-three minutes (stable total coefficient of variation =1%). Light response curves were started at a PFD of 2000 µmol/m^2/s and then decreased to 1800, 1500, 1200, 900, 600, 300, 100, 75, 50, 25, 10, and 0 µmol/m^2/s.

Measurements included net photosynthesis, stomatal conductance, and transpiration, which were measured over 13 light levels (see above). A one-way ANOVA was used to determine if net photosynthesis, stomatal conductance, and transpiration were significantly different over the PFD's tested (Sall et al. 2001). The Shapiro-Wilks test was used to test for normal distributions and Bartlett's Test was

used to test for homogeneity of variances. If unequal variances were detected, data were log transformed prior to analyses.

Data presented in the figures were taken directly from the LI-COR©6400. Tabular results were adjusted for each replication (plant) and fitted to the model of Prioul and Chartier (Prioul and Chartier 1977) using the PC software package Photosyn Assistant (Dundee Scientific, Dundee, Scotland). Fitted data included A_{max} (maximum photosynthesis)(μmol·CO_2/m^2/s), PFD at A_{max} (μmol/m^2/s), transpiration at A_{max} (μmol·H_2O/m^2/s), conductance at A_{max} (mmol·H_2O/m^2/s), light saturation point (μmol/m^2/s), dark respiration (μmol·CO_2/m^2/s), light compensation point (μmol/m^2/s), and the quantum yield efficiency (μmol·CO_2/μmol quanta). Values were calculated for each replicate and then averaged. The highest net photosynthesis rate was the A_{max}. The PFD when the slope of the initial rate line reached the A_{max} was light saturating photosynthesis. The gas exchange rate at a PFD of zero μmol/m^2/s (y-intercept of the line for the initial rate) was dark respiration. The light compensation point was calculated as the PFD when the photosynthetic rate was zero μmol CO_2/m^2/s (x-intercept of the line for the initial rate). The quantum yield efficiency was determined by using the dark value and increasing PFDs until the regression coefficient of the slope decreased. An alpha value of 0.05 was used for all tests. Tukey-Kramer HSDs were used to detect significant differences between gas exchange rates at each PFD (Sall et al. 2001).

Ambient CO_2 uptake--CO_2 uptake and other gas exchange parameters (see above) were also made at ambient light, temperature, and humidity levels in the field below the canopy with the soil at field capacity. Measurements were made ± 2 hr. of solar noon on a clear sunny day (30 April 2012). We used the clear window (2 X 3 cm) for making these measurements and leaf material covered the entire surface area of the window. Ten replicate plants from the same population as above were measured with five replicate measurements per leaf or plant (total=50). Measurements were averaged to get mean and standard deviations for all parameters and compared with the gas exchange rates at specific light levels of the light response curves previously measured.

RESULTS

Size and density of four *Chaptalia texana* populations were examined (Table 1). All *C. texana* plants found were below the canopy of *Juniperus-Quercus* woodlands with no *C. texana* plants found in the adjacent C_4 grassland phase of the savanna. Mean area (± SD) occupied by the populations was 175 ± 84 m^2. The mean number of *C. texana* plants in the populations was 97 ± 41 and they had a mean density of 0.57 ± 0.05 plants/m^2. In the community with the greatest number of plants (Fig. 1), the distribution of *C. texana* appears to be clumped or non-random with density varying from zero to15 plants/m^2.

Light levels were highest in the open grassland on clear, sunny, spring days versus cloudy days (Fig. 2, Table 2), and reduced below the *Juniperus-Quercus* canopy. Light levels in the open over the whole diurnal period on a sunny spring day were 1129 ± 659 μmol/m^2/s and were reduced 52–63% to 651 ± 360 μmol/m^2/s on cloudy days. Over the diurnal period, mean light levels increased to a maximum of approximately 2100 μmol/m^2/s on clear, spring days near 1400 hr. (Fig. 2A, Table 2). Below the canopy, light levels were quite variable and reduced to 20-26% on sunny days relative to adjacent open areas (Table 2). On cloudy, spring days, light levels in the open were more variable (Fig. 2B, Table 2), reaching high or low values more quickly and remaining at these values for shorter times. Light levels below the canopy on cloudy days were reduced to about the same percentage as on clear, sunny days, but actual light levels were between approximately 200 and 300 μmol/m^2/s during the brightest part of the day.

The mean CO_2 uptake for the photosynthetic light response curve for leaves of plants grown in shade was significantly different over the light levels measured (one-way ANOVA, $F=107.7$, $P < 0.0001$; Fig. 3). At PFD's between 300-2000 μmol/m^2/s uptake rates were fairly constant, with few significant differences and many of the rates overlapping. At the highest light level tested, the A_{max} was 19.24 ± 1.23 μmol·CO_2/m^2/s (Table 3). At light levels (PFD's) below approximately 300 μmol/m^2/s there was a rapid decline in the photosynthetic rates with few significant differences (Fig. 3).

Dark respiration (R_d) for *C. texana* was 1.18 ± 0.16 μmol·CO_2/m^2/s (Table 3) and the quantum yield efficiency or the initial slope was 0.081 ± 0.004 μmol·CO_2/μmol quanta. Mean light saturation (L_{sat}) for *C. texana* was 254 ± 22 μmol/m^2/s and the light compensation point (L_{cp}) was 15 ± 2 μmol/m^2/s (Table 3).

Mean stomatal conductance (g_{leaf}) was significantly different over the light levels examined (one-way ANOVA, F=12.9, $P < 0.001$). Significant differences in stomatal conductance were at the highest and lowest light levels tested. The lowest rate, 0.082 mol·H_2O/m^2/s, was at the zero light level. Stomatal conductance was highest at 0.379 mmol·H_2O/m^2/s at the highest light level tested, the A_{max} (Table 3). Transpiration rates were also significantly different over the light levels measured (one-way ANOVA, F=12.5, $P = 0.001$). However, differences were significant only at the highest and the lowest light level tested. Lowest transpiration was 2.201 mmol·H_2O/m^2/s at zero PFD and the highest value was 6.902 ± 0.828 mmol·H_2O/m^2/s at the A_{max} (2000 μmol/m^2/s) (Table 3).

Ambient CO_2 uptake was also measured in the field on leaves of *C. texana* growing below the *Juniperus-Quercus* canopy. Mean CO_2 uptake rates were 4.92 ± 0.72 μmol·CO_2/m^2/s at ambient light levels of 143 ± 50 μmol/m^2/s. Carbon dioxide uptake rates were similar to those measured for the light response curves at the ambient light level (Fig. 3). Relativity humidity and air temperature at the time of measurement (1000-1400 hrs.) were 33.6 ± 3.1% and 32.4 ± 1.9°C (30 April 2012, Table 4). Conductance and transpiration at ambient PFD were 0.086 ± 0.026 mol·H_2O/m^2/s and 2.394 ± 0.566 mmol·H_2O/m^2/s.

DISCUSSION

Herbaceous species found below forest canopies typically have gas exchange rates that are low and if exposed to light levels characteristic of open grassland communities, their gas exchange rates do not usually increase greatly (Zangerl and Bazzaz 1983; Hättenschwiler and Körner 1996; Hirose and Bazzaz 1998; Hull 2002)(see Table 3 for comparisons). However, species from central Texas savannas have mixed responses to light levels. *Carex planostachys*, a drought tolerant sedge found mainly below the *Juniperus-Quercus* canopy had low gas exchange rates similar to true forest understory shade species (Wayne and Van Auken 2012). A series of C_4 grasses (sun species) from open habitats in this same area had high gas exchange rates at high light levels (Wayne and Van Auken 2012). A leguminosae, shrub, four asteraceae and all malvaceae sub-shrubs had intermediate levels of gas exchange and were considered facultative species found below the canopy, at the canopy edge and sometimes in open areas (Furuya and Van Auken 2009; Gagliardi and Van Auken 2009; Furuya and Van Auken 2010; Van Auken and Bush 2011). These species were able to modify their gas exchange rates depending on the light levels where they were growing.

Chaptalia texana was found in low light environments below the canopy of *Juniperus-Quercus* woodlands in Central Texas savannas, not in open grasslands. Gas exchange rates for *C. texana* below the canopy at low light levels were equivalent or a little higher than rates measured for true understory species and rates measured for *C. texana* in light response curves at the same light levels (Table 4, Fig. 3). These rates should make *C. texana* a good competitor with other understory species in this same understory environment. Interestingly, gas exchange rates for *C. texana* at higher light levels were typical of species of open not understory habitats (Begon et al. 2006) (see Table 3). Other photosynthetic parameters, including light saturation, light compensation, dark respiration, conductance, and transpiration, were high as well (see Table 3). These responses are not consistent with findings for shade plants, but for sun plants or intermediate or facultative species (Boardman 1977; Hull 2002; Larcher 2003; Givnish et al. 2004; Begon et al. 2006; Valladares and Niinemets 2008). The parameters measured for shade adapted leaves of *C. texana* at elevated light levels suggest that this species is not a true understory species but a facultative species, one capable of growth in high light environments like disturbances in open grasslands.

In general, true understory species or shade species have much lower A_{max} values and photosynthetic rates than the rates reported for *C. texana* in the current study (see Table 3). No *C. texana* plants were found in full sun, consequently we do not know if they could acclimate to a variable light environment as reported for other species (Hull 2002; Valladares and Niinemets 2008).

Dark respiration of shade leaves of *C. texana* (1.18 ± 0.16 μmol·CO_2/m^2/s) was about 33% of values for sun-adapted species (Hamerlynck and Knapp 1994)(Table 3). This rate for *C. texana* is about 50% higher than the R_d of leaves of shade adapted species (Hirose and Bazzaz 1998; Hull 2002). Dark respiration for shade-adapted species is typically low due to their lower metabolism (Bjorkman 1968; Bazzaz and Carlson 1982). *Polygonum pensylvanicum* grown at 200 μmol/m^2/s had a respiration rate of ~ 0.5 μmol·CO_2/m^2/s, while the rate for its sun leaves was twice as high (Bazzaz and Carlson 1982).

Other gas exchange values reported in this study for *C. texana* are similar to those reported for sun plants or sun leaves from the literature (Table 3). For example, the quantum yield efficiency reported here was 0.081 ± 0.004 μmol·CO_2/μmol quanta, for shade leaves which is 37% higher than values reported for other shade species (0.035 – 0.052 μmol·CO_2/μmol quanta) (Hirose et al. 1997). This may be a rapid response to light flecks below the canopy (Fig. 2), but this is speculation at this time. Stomatal conductance and transpiration reported for *C. texana* in the current study were similar to other studies and indicate open stomates; however, many factors affect the levels of these parameters including temperature and soil water content (Wieland and Bazzaz 1975; Zangerl and Bazzaz 1984; Yun and Taylor 1986; Munger et al. 1987a; Munger et al. 1987b; Stafford 1989).

The high gas exchange rates and A_{max} values reported for light response curves of shade leaves of *C. texana* present a conundrum. The reason being that all of the plants we found were below the *Juniperus-Quercus* canopy and all had high A_{max} values when leaves were exposed to high light levels. We never found plants of this species or associated species in open grasslands. Descriptions of this species suggest it is a canopy adapted species (Correll and Johnston 1979; Enquist 1987; Nesom 1995; Flora of North America 2003). Earlier work suggests that the various species of *Chaptalia* are found below *Quercus* or *Pinus-Quercus* woodlands or savannas (Nesom 1984; 1995; Flora of North America 2003) and *Juniperus-Quercus* woodlands and savannas (Harms 2011). In parts of the range of the genus *Chaptalia*, some species or individuals of all species of *Chaptalia* may establish and grow in low density grasslands outside of the woodland canopy. However, all of the *C. texana* plants that we found were below the *Juniperus-Quercus* woodland canopy.

When a species is found in a given habitat, it can tolerate or requires the environmental conditions present in that habitat. Conditions where it is found may be optimal for its growth and survival. However, sorting out the characteristics or factors that determine why a species is present in a given habitat and not in other habitats is challenging (Begon et al. 2006; Smith and Smith 2012). We believe that while *C. texana* is usually found growing in shade, gas exchange characteristics are not the factor controlling its apparent habitat preference. We would like to further evaluate the drought tolerance of this species to determine if it is restricted to areas below the canopy because it cannot compete with the drought tolerant C_4 grasses growing in the open. Another environmental factor or a combination of factors may limit the growth of *C. texana* to shaded understory habitats including possible photo-inhibition of leaf pigments or overheating of leaves (Begon et al. 2006). Similar patterns have been reported for other species, but restrictions were caused by herbivory (Louda and Rodman 1996; Maron and Crone 2006; Leonard and Van Auken 2013).

Soil water may be a resource limiting *C. texana* because of water use by more drought tolerant C_4 grasses, which seems to keep *C. texana* restricted to canopy habitats where these grasses cannot grow and compete because of low light levels and their high light requirements (Wayne and Van Auken 2009) or

because of higher soil water levels below the canopy available to *C. texana* or because of some combination of the two factors.

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Table 1. Mean area (± one standard deviation) of the four *C. texana* populations studied, the mean number of plants counted per population and the density of plants/m^2 in the populations. Measurements were below woodland canopies and in adjacent open grasslands.

	Mean Area of population (m^2)	Number of plants	Density (plants/m^2)
Woodland	175 ± 84	97 ± 41	0.57 ± 0.05
Grassland	---*	---	---

* --- no plants were found

Table 2. Mean ± one standard deviation of the photosynthetic-flux density (PFD, µmol/m^2/s) at various time intervals measured in the open or below the woodland canopy and percent values of light levels reaching the *C. texana* plants at those times and light conditions.

	Sunny day			Cloudy day		
Time	Open	Below canopy	%	Open	Below canopy	%
All Daylight Hours	1129 ± 659	222 ± 195	20	651 ± 360	138 ± 80	21
10:00-17:00	1857 ± 269	371 ± 238	20	968 ± 261	210 ± 66	22
12:15-15:45	1982 ± 153	464 ± 297	23	1129 ± 214	260 ± 64	23
12:45-14:45	2098 ± 41	545 ± 86	26	1102 ± 190	288 ± 95	26

Table 3. Physiological measurements of *C. texana* leaves taken from light response curves (means ± one standard deviation, SD). Plants were growing below a *Juniperus-Quercus* woodland canopy. Ranges of gas exchange rates for a number of sun and shade species are also presented.

Parameter	Sun species-range	Shade species-range	*Chaptalia*
A_{max} – Max. gas exchange (µmol·CO_2/m^2/s)	15.0-34.6*	3.5-7.0*	19.24 ± 1.23**
Photosyn. flux density at A_{max} (µmol/m^2/s)	1300-2000	700-1300	2000 ± 0.0
R_d - Dark Respiration (µmol·CO_2/m^2/s)	2.4-3.0	0.29-1.00	1.18 ± 0.16
Qe - Initial slope-quantum yield	0.059-0.090	0.070-0.090	0.081 ± 0.004
L_{sat} - Mean light saturation (µmol/m^2/s)	326-1000	114-262	254 ± 22
L_{cp} - Light compensation point (µmol/m^2/s)	13-61	3-21	15 ± 2
g_{leaf} – Conductance (mol·H_2O/m^2/s) at A_{max}	-	-	0.379 ± 0.077
Conductance range (mean)	0.13-0.60	0.05-0.36	0.082-0.379
E_{leaf} – Transpiration (mmol·H_2O/m^2/s) at A_{max}	-	-	6.902 ± 0.828
Transpiration range (mean)	7.1-12.1	0.9-3.9	2.201-6.902

*From Van Auken and Bush 2009, Hull 2002, Wayne and Van Auken 2012, Valladares and Niinemets 2008; **Mean ± one SD

Table 4. Gas exchange and physical measurements (means ± one SD) for leaves of *C. texana* plants made in the field. Measurements were made on 30 April 2012, a clear day, between 1100 and 1300 hours. The clear window chamber was used with ambient light levels.

Parameter	*Chaptalia texana*
CO_2 uptake at ambient light level (μmol·CO_2/m^2/s)	4.92 ± 0.72
Ambient Photosynthetic-flux density PFD (μmol/m^2/s)	143 ± 50
Relative Humidity (%)	33.6 ± 3.1
Air Temperature (°C)	32.4 ± 1.9
Leaf Temperature (°C)	30.6 ± 0.7
g_{leaf} – Conductance (mol·H_2O/m^2/s) at Ambient PFD	0.086 ± 0.026
E_{leaf} – Transpiration (mmol·H_2O/m^2/s) at Ambient PFD	2.394 ± 0.568

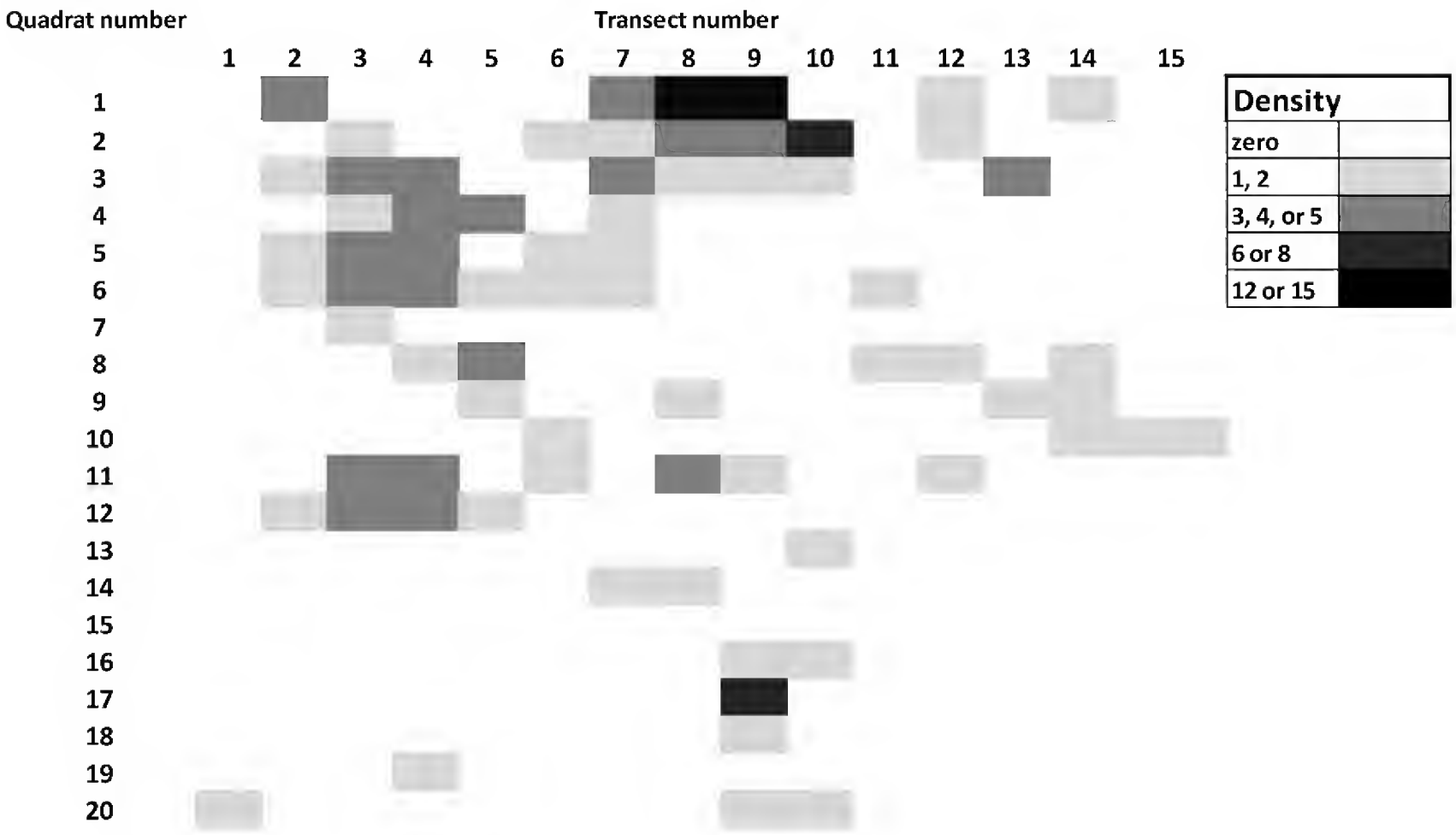

Figure. 1. Example distribution and density of the largest population of *C. texana* plants found growing below a *Juniperus-Quercus* woodland in central Texas. Blocks represent one meter square. Density ranged from zero plants/m^2 (white or clear blocks) to 12-15 plants/m^2 (black blocks). Plants appear to be clumped in distribution, but the reason was not investigated.

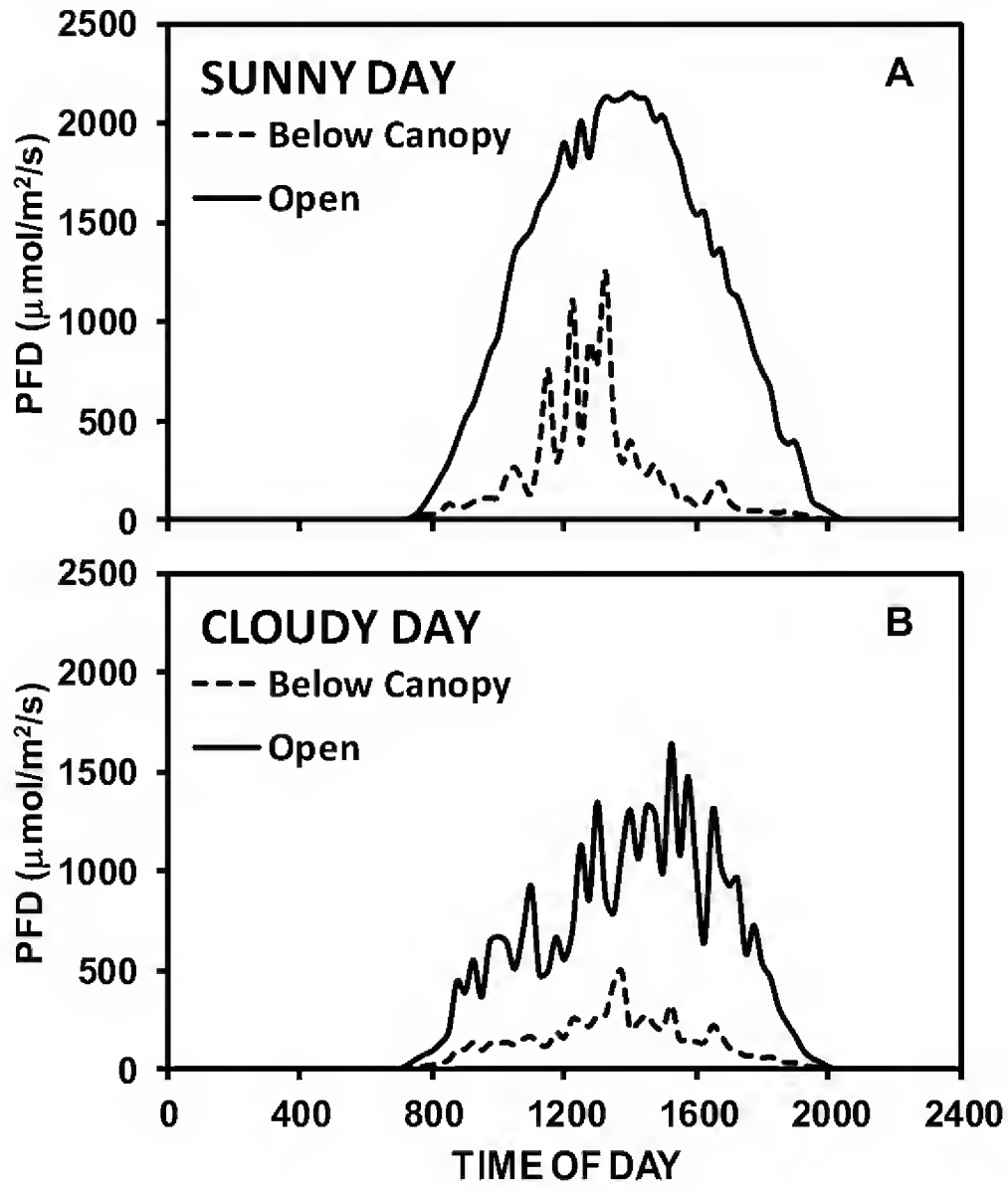

Figure 2. Mean diurnal light levels (PFD = Photosynthetic Flux Density, $\mu M/m^2/s$) are shown for an open grassland or glade (solid line) and below a *Juniperus-Quercus* woodland canopy (dashed line) for a clear spring day (A) and an overcast, cloudy spring day (B).

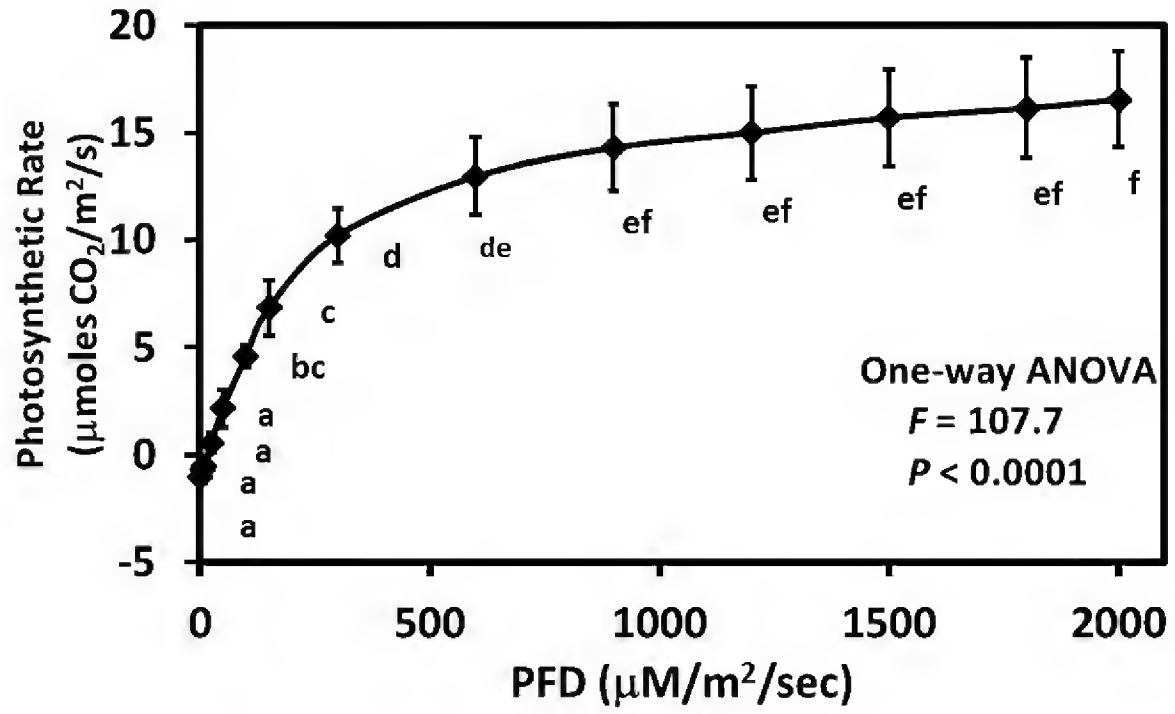

Figure 3. Gas exchange rates (photosynthetic rates, CO_2 uptake) for leaves of *C. texana* growing below a *Juniperus-Quercus* woodland canopy as a function of light levels or photosynthetic flux density (PFD, $\mu M/m^2/s$). There were significant differences in photosynthetic rates at different light levels (one-way ANOVA, $P < 0.0001$). Error bars represent ± one standard deviation of the mean. Same letters between light levels indicate no significant differences between light levels (Tukey-Kramer HSD, $P > 0.05$).

First comprehensive report on the composition of the leaf volatile terpenoids of *Pinus arizonica* Engelm. and *P. ponderosa* var. *brachyptera* (Engelm.) Lemmon

Robert P. Adams
Biology Department, Baylor University, Waco, TX 76798 robert_adams@baylor.edu

George M. Ferguson
Herbarium, Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ 85721

and

David Thornburg
2200 W. Winchester Lane, Cottonwood AZ 86326

ABSTRACT

The first comprehensive report on the composition of the volatile leaf terpenoids of *Pinus arizonica* and *P. ponderosa* var. *brachyptera* from Arizona is presented. The leaf oil of *P. arizonica* contains major amounts of α-pinene (19.4%), germacrene D (19.0%), (E)-caryphyllene (10.1%) and β-phellandrene (9.0%) with moderate amounts of β-pinene (5.0%) and limonene (4.4%). The leaf oil of *P. p.* var. *brachyptera* has considerable α-pinene (15.2%), germacrene D (13.6%), β-pinene (11.2%), (E)-caryphyllene (9.7%) and δ-3-carene (9.2%), with modest amounts of β-phellandrene (2.4%), limonene (2.3%),terpinolene (2.1%), camphene(2.1%), myrcene(2.1%), bornyl acetate(2.0%) and (Z)-β-ocimene(1.8%). The oil of *P. arizonica* contains seven unique compounds: longifolene, unknown sesquiterpene (KI 1494), ethyl dodecanoate, ethyl tetradecanoate, iso-abienol, abienol and unknown diterpene (KI 2341). The oil of *P. p.* var. *brachyptera* has eight unique compounds: δ-3-carene, 2-undecanone, β-longipinene, β-himachalene, α-cuprenene, unknown sesquiterpene aldehyde (KI 1756), putative (2E)-tridecenol, and phenyl ethyl octanoate. Published on-line **www.phytologia.org** *Phytologia 97(1): 45-50 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: *Pinus arizonica*, *Pinus ponderosa* var. *brachyptera*, volatile leaf oil, terpenes, composition.

Smith (1977) reported extensively on five major monoterpenes in the xylem resin and found five types of wood oils in ponderosa pine (Table 1). Notice that the combination of the five monoterpenes characterizes each of the five types. However, types IV and V are not very distinct. Type V (Chiricahua,

Table 1. Average normalized monoterpene composition in xylem resin from naturally growing trees. (data from Smith, 1977, Table 4) arranged by the five oil types (I, II, III, IV, V) of Smith (1977).

compound	San Bernardino Type: I	Eldorado, CA II	Pierce, WA III	Coconino, AZ IV	Coronado, AZ V (*P. arizonica*)
α-pinene	9.1	6.1	4.8	50.4	69.9
β-pinene	46.2	28.3	11.8	4.2	6.9
δ-3-carene	4.2	34.5	59.5	26.9	12.0
myrcene	13.7	13.1	13.6	4.3	1.4
limonene	24.8	14.9	6.5	11.2	4.6

AZ) is currently treated as *P. arizonica* and type IV (Coconino, AZ) is in the range of *P. ponderosa* var. *brachyptera*. Potter et al. (2013) did not find many differences in mitochondria haplotypes, although it is unclear if they sampled typical *P. arizonica*. Gernandt et al. (2009, fig. 2) also found only a few inconsistent cpDNA differences between *P. arizonica* and *P. ponderosa* (*P. scopulorum*).

Smith (1977) did not find differences in the xylem monoterpenes between the northwestern ponderosa pine (*P. p.* var. *ponderosa*) and the northeastern populations (*P. p.* var. *scopulorum*). However, von Rudloff and Lapp (1992), using leaf essential oils, found var. *ponderosa* and var. *scopulorum* to clearly differ in leaf oil composition. Potter et al. (2013) confirmed these varieties in their mitochondrial DNA study.

Although there are scores of studies on the xylem monoterpenes (see review in Smith, 1977), there are very few studies on the volatile leaf oil composition of *P. ponderosa*. The earliest report was by Schorger (1919) that the leaf oil was composed of α-pinene (2%), β-pinene (75%), limonene (6%), borneol (7%), bornyl acetate (2%), and 'green oil' (3%). In the modern era of gas chromatography/ mass spectrometry, Zavarin et al. (1971) reported on the variation in the leaf oil with season and needle age. Cobb et al. (1972) reported the effects of pollution on the volatile leaf oils. Von Rudloff (1975) gave a bar histogram of 12 components from *P. ponderosa* (var. *ponderosa*) from British Columbia.

The first comprehensive analysis of the volatile leaf oil of *P. ponderosa* (var. *ponderosa*), was by Adams and Edmunds (1989) from 20 trees near Dryden, WA. The only major study on geographical variation in the volatile leaf oils of *P. ponderosa* was by von Rudloff and Lapp (1992). They examined the leaf oils from 37 populations west of the Continental Divide and 5 populations east of the Divide in MT using 12 terpenoids plus estragole and 5 consolidated characters (composed of difficult to resolve, isomers). They found the terpenoids clearly distinguished the western (var. *ponderosa*) from the eastern (var. *scopulorum*) populations. However, there appear to be intergradation between the two varieties in the terpene patterns near the Continental Divide. In addition, von Rudloff and Lapp (1992) analyzed one tree from Grand Canyon, AZ (presumably *P. ponderosa* var. *brachyptera*) and reported the oil was dominated by α-pinene (37.0%), β-pinene (18.4%), δ-3-carene (6.9%), limonene (6.5%),

The purpose of the present paper is to make the first comprehensive report on the leaf volatile oil of *P. arizonica* from near the type locality in far southern Arizona and compare it with the leaf oil of *P. ponderosa* var. *brachyptera* from northern Arizona.

MATERIALS AND METHODS

Leaf samples were collected from *Pinus arizonica:* Coronado National Forest, Santa Catalina Mountains, Bear Canyon, 0.2 mi WNW Cypress picnic area, 32.3732° N, 110.6975° W, elev. 5900 ft. Pima Co., AZ, *G. M. Ferguson 3679-3687,* lab acc. *Robert P. Adams 14439-14447*; Coronado National Forest, Santa Catalina Mountains, Bear Canyon, 0.1 mi E Cypress picnic area, same tree visited previously with David Gernandt in 2007, his DNA analysis. 32.3729° N, 110.6923° W, elev. 5870 ft., Pima Co., AZ, *G. M. Ferguson 3688,* lab acc. *Robert P. Adams 14448*.

Pinus ponderosa var. *brachyptera*: 19 mi. east of Camp Verde on AZ Hwy 260 at Salmon Lake, Coconino National Forest. 34° 30' 38.5" N, 111° 31' 28.1" W. elev. 6,300 ft, Coconino, Co., AZ, *David Thornburg 1419 (1-10)*, lab acc. *Robert P. Adams 14428-14437.* Voucher specimens are deposited in the herbarium, Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The leaf oil of *P. arizonica* contains major amounts (Table 2) of α-pinene (19.4%), germacrene D (19.0%), (E)-caryphyllene (10.1%) and β-phellandrene (9.0%) with moderate amounts of β-pinene (5.0%) and limonene (4.4%). The leaf oil of *P. p.* var. *brachyptera* has considerable α-pinene (15.2%), germacrene D (13.6%), β-pinene (11.2%), (E)-caryphyllene (9.7%) and δ-3-carene (9.2%), with modest amounts of β-phellandrene (2.4%), limonene (2.3%),terpinolene (2.1%), camphene (2.1%), myrcene (2.1%), bornyl acetate (2.0%) and (Z)-β-ocimene (1.8%). The oil of *P. arizonica* contains seven unique compounds: longifolene, unknown sesquiterpene (KI 1494), ethyl dodecanoate, ethyl tetradecanoate, iso-abienol, abienol and unknown diterpene (KI 2341). The oil of *P. p.* var. *brachyptera* has eight unique compounds: δ-3-carene, 2-undecanone, β-longipinene, β-himachalene, α-cuprenene, unknown sesquiterpene aldehyde (KI 1756), putative (2E)-tridecenol, and phenyl ethyl octanoate.

Pinus arizonica individual 14443 is unusual in having larger amounts of limonene (11.0%) and β-phellandrene (22.2%) but low in α-pinene (7.5%). Tree 14442 is high in α-pinene (31.9%) and low in limonene (0.4%) and β-phellandrene (0.8%). However, the unique components that separate the two taxa are still present (or absent) in these unusual tree oils (Table 2).

Likewise, *P. p.* var. *brachyptera* has trees with unusual oils: 14430 is low in monoterpenes and high in linalyl acetate (8.5%), germacrene D (21.5%) and (E)-caryphyllene (11.7%); trees 14433 (and 14436, not shown) are also low in monoterpenes, but high in bornyl acetate (10.6%), (E)-caryphyllene (13.9%) and germacrene D (14.5%). Just as with *P. arizonica*, these trees, with unusual oils, still have the unique components that separate the two taxa or the compounds are absent in their oils as in the average (Table 2).

Overall the leaf oils differ about as expected between conifer species. In any case, the differences in the morphology clearly distinguish these taxa.

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Table 2. Comparison of leaf oil compositions of *Pinus arizonica and P. ponderosa* var. *brachyptera.* Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear calculation on DB-5 column.

		P. arizonica			*P. ponderosa brachyptera*		
KI	compound	*14443*	*14442*	average	average	*14430(1)*	*14433(2)*
846	(E)-hexenal	0.6	0.1	0.3	0.1	t	0.4
850	(3Z)-hexenol	0.4	0.1	0.3	t	t	0.3
863	n-hexenol	0.1	t	t	t	t	t
921	tricyclene	0.1	0.2	0.1	0.3	t	0.5
924	α-thujene	t	-	t	0.2	t	0.2
932	α-pinene	7.5	31.9	19.4	15.2	7.9	7.2
946	camphene	0.6	0.5	1.1	2.1	0.6	1.8
969	sabinene	0.4	t	0.1	0.2	0.2	0.2
974	**β-pinene**	**3.5**	**0.5**	**5.0**	**11.2**	**1.3**	**6.8**
988	myrcene	1.7	0.7	1.4	2.1	2.5	1.8
1002	α-phellandrene	1.0	t	0.4	0.2	t	0.1
1008	**δ-3-carene**	**-**	**-**	**-**	**9.2**	**4.3**	**7.5**
1014	α-terpinene	0.3	0.2	0.8	1.0	0.8	0.5
1020	p-cymene	0.1	t	t	0.3	0.2	0.2
1024	**limonene**	**11.0**	**0.4**	**4.4**	**2.3**	**1.7**	**0.9**
1025	**β-phellandrene**	**22.2**	**0.8**	**9.0**	**2.4**	**1.8**	**1.1**
1032	**(Z)-β-ocimene**	**1.7**	**0.6**	**0.4**	**1.8**	**1.6**	**1.0**
1044	(E)-β-ocimene	0.2	0.8	1.6	0.7	0.2	0.1
1054	γ-terpinene	0.2	0.1	0.2	0.5	0.4	0.4
1086	terpinolene	0.4	0.6	0.8	2.1	1.6	1.4
1095	linalool	0.4	0.1	0.1	1.2	0.9	3.7
1122	α-campholenal	0.1	0.1	0.1	t	t	0.1
1135	trans-pinocarveol	0.3	0.1	0.1	0.1	t	0.2
1137	cis-verbenol	0.1	0.1	0.1	-	t	-
1166	p-mentha-1,5-dien-8-ol	t	0.1	t	0.2	t	0.8
1174	terpinen-4-ol	0.2	t	t	0.1	t	0.2
1186	**α-terpineol**	**0.3**	**0.2**	**0.2**	**0.7**	**0.4**	**0.7**
1254	linalyl acetate	2.2	0.8	1.1	1.2	8.5	2.8
1287	bornyl acetate	0.8	0.4	0.6	2.0	0.3	10.6
1298	**trans-pinocarvyl acetate**	t	t	**t**	**0.4**	t	0.3
1345	α-terpinyl acetate	0.1	0.4	t	0.2	0.8	1.0
1345	α-cubebene	0.1	0.1	t	0.1	0.1	0.2
1365	**2-undecanone**	**-**	**-**	**-**	**0.1**	**0.3**	**0.2**
1373	α-ylangene	0.1	t	t	t	t	t
1374	α-copaene	0.1	0.2	0.2	0.3	0.4	0.4
1379	geranyl acetate	t	t	t	-	t	-
1387	β-bourbonene	t	0.1	t	-	0.2	-
1387	β-cubebene	t	0.1	t	t	0.1	0.2
1400	**β-longipinene**	**-**	**-**	**-**	**t**	**0.4**	**0.9**
1407	**longifolene**	**2.2**	**t**	**0.3**	**-**	**-**	**-**
1417	(E)-caryophyllene	7.1	13.4	10.1	9.7	11.7	13.9
1430	β-copaene	0.1	0.2	0.2	t	0.1	0.1
1448	cis-muurola-3,5-diene	t	t	t	t	t	t
1451	trans-muurola-3,5-diene	t	t	t	t	t	t
1452	α-humulene	1.1	2.2	1.6	1.6	1.9	2.2
1461	cis-cadina-1(6),4-diene	t	t	t	t	t	t
1465	cis-muurola-4(14),5-diene	t	t	t	t	t	t
1475	γ-muurolene	0.7	2.2	2.0	0.5	2.2	0.7
1478	germacrene D	12.7	23.2	19.0	13.6	21.5	14.5
1475	trans-cadina-1(6),4-diene	t	t	t	t	0.1	0.1
1493	trans-muurola-4(14),5-diene	t	t	t	t	0.1	0.3
1494	**sesquiterpene,161,119,105, 204**	**0.5**	**0.5**	**0.5**	**-**	**-**	**-**
1500	**β-himachalene**	**-**	**-**	**-**	**1.1**	**1.6**	**1.0**
1500	α-muurolene	0.9	0.9	1.0	0.8	1.3	0.8
1505	**α-cuprenene**	**-**	**-**	**-**	**0.2**	**0.4**	**0.1**
1511	δ-amorphene	**-**	**-**	0.1	-	**-**	**-**
1513	γ-cadinene	1.5	1.1	1.3	1.4	1.8	1.1

		P. arizonica			*P. ponderosa brachyptera*		
KI	compound	*14443*	*14442*	average	average	*14430(1)*	*14433(2)*
1522	δ-cadinene	3.3	2.3	3.0	2.4	3.4	1.8
1533	trans-cadina-1,4-diene	0.1	0.1	t	t	0.2	0.1
1537	α-cadinene	0.2	0.1	0.2	0.1	0.2	0.1
1561	(E)-nerolidol	t	0.2	t	0.9	0.8	0.7
1574	germacrene-D-4-ol	5.5	3.5	3.9	2.7	5.0	0.4
1582	caryophyllene oxide	0.3	0.3	0.3	0.2	0.2	0.3
1594	**ethyl dodecanoate**	**0.7**	**0.3**	**0.5**	**-**	**-**	**-**
1638	epi-α-cadinol	0.8	0.4	0.6	0.6	1.0	0.1
1640	epi-α-muurolol	0.8	0.5	0.7	0.6	0.9	0.2
1644	α-muurolol	0.3	0.2	0.2	0.2	0.4	0.1
1652	α-cadinol	2.3	1.3	1.8	1.7	2.7	0.5
1756	**hydrocarbon-aldehyde 55, 43, 69, 121**	**-**	**-**	**-**	**0.8**	**0.6**	**0.7**
1772	**(2E-tridecenol?)**	**-**	**-**	**-**	**0.7**	**0.5**	**0.7**
1795	**ethyl tetradecanoate**	**0.2**	**0.2**	**0.1**	**-**	**-**	**-**
1846	**phenyl ethyl octanoate**	**-**	**-**	**-**	**0.5**	**1.9**	**0.1**
1987	manool oxide	0.1	0.3	0.5	0.3	t	t
2105	**iso-abienol**	**-**	**-**	**0.9**	**-**	**-**	**-**
2149	**abienol**	**0.1**	**0.3**	**0.5**	**-**	**-**	**-**
2341	**diterpene,55,81,239,286**	**0.9**	**0.2**	**1.0**	**-**	**-**	**-**

Oryctanthus callicarpus, a replacement for Mesoamerican *O. occidentalis* (Loranthaceae).

Job Kuijt
649 Lost Lake Road, Victoria, BC V9B6E3, Canada
jobkuijt@uvic.ca

and

Jerry and Linda Harrison
PTY 9617, 2250 NW 114th Ave., Unit 1P, Miami, Fl 33172, USA

ABSTRACT

A new species name, *Oryctanthus callicarpus*, is proposed to replace *O. occidentalis* subsp. *continentalis*. The species is characterized by complexly-banded fruits having strongly truncate apices, in contrast to the Jamaican *O. occidentalis*, that has blackish purple fruits with rounded apices. Published on-line **www.phytologia.org** *Phytologia 97(1): 51-54 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: *Oryctanthus callicarpus*, *Oryctanthus occidentalis*, Mesoamerica, Loranthaceae, replacement.

The name *Oryctanthus occidentalis* (L.) Eichler has traditionally been applied to two separate regions, the type locality (Jamaica; see Kuijt & Zanoni 2013 for corrected typification) and the continental area from Mesoamerica to Peru (Kuijt 1976). It has been recognized for some time that the plants of the two areas might well show differences sufficiently significant to warrant subspecific separation. This separation was more recently formalized in the recognition of *O. occidentalis* subsp. *continentalis* Kuijt for the continental plants (Kuijt 1992). The contrast in fruit color was the major reason for that proposal, the Jamaican plants having blackish purple fruits (Fawcett & Rendle 1914) and those of the new subspecies "green or green and yellow (red)" fruits (Kuijt 1976). In the latter paper it was noted that the Andean area showed more structural variation than the other continental areas. A particularly striking element of variation, not fully explored at the time, is the variation in the number of functional pollen sacs on the stamens, between collections and even within some flowers.

Recent field experience in the Cerro Jefe area of Panama, combined with a partial review of herbarium material from that country, has demonstrated the inadequacy of previous solutions. Extending our studies into the Costa Rican and variable South American populations, unfortunately, is not possible at this time. Nevertheless, our findings especially with regards to the unique, striking multicolored fruits in Panama, features not normally surviving in herbarium material, warrant the description of a new species. Two previous publications have already hinted at this color variation (Croat 1978, Kuijt 1976). We may speculate that a detailed, South American analysis of this, the most complex species in the genus, may lead to further changes, but it will likely continue to restrict the application of the name *O. occidentalis* to Jamaica. The illustration of the species for the Flora of Ecuador (Kuijt 1986: 123), for example, shows a fruit type different from both Jamaican and Panamanian fruits.

Oryctanthus callicarpus Kuijt, *nom. nov* . -- *Oryctanthus occidentalis* (L.) Eichler subsp. *continentalis* Kuijt (1972) 181, *syn. nov.* -- Type: Costa Rica. Puntarenas, Palmar Sur, on *Citrus* sp., *J. Kuijt 2570* (holotype CR; isotype UBC).

Diagnosis. *Oryctanthus callicarpus*, uniquely in Loranthaceae, has fruits with a variable number of transverse color bands, being barrel-shaped with abruptly truncate apices. Fruits are densely crowded on the infructescences.

Description. Dark green, percurrent, glabrous plants with epicortical roots at the base. Internodes terete, grayish brown, with innumerable, minute, light brown lenticels, commonly 4--8 cm long. Leaf blade to 12(15) cm long and 9(13) cm wide, broadly ovate to elliptical, sometimes nearly orbicular, base obtuse to nearly truncate or slightly acute, apex rounded, leaf margin callused, gray; venation pinnate or nearly so, with 3--5 prominent veins, these often purplish red below; petiole 4--5 mm long. Inflorescences axillary, the primary one often with two or more secondary ones; length of inflorescence 1--11 cm long, peduncle 8--15 mm long, terete, followed by 20 or more decussate pairs of flowers, one flower in each axil of an inconspicuous green, scale-like bract, bracteoles minute, black, scarcely reaching the margin of the floral cup; floriferous axis dark green between floral series, glabrous and shiny. Flowers partly sunken in rachis, deep reddish-purple, up to 120 per inflorescence, commonly ca. 4 cm long with about 60 flowers (for floral details, see Kuijt, 1976). Fruits barrel-shaped, 3 x 3 mm, densely crowded together in each series, variably colored but often with multicolored rings, the base reddish, and followed by rings of dark green and yellow, apex truncate, nectary area green; some plants lacking red either completely or having red only as the distal ring. Sea level to ca. 1000 m. Fig. 1.

Representative specimens examined. COSTA RICA. **Cartago**: Murray's Finca, Cachí, on *Psidium guajava* and others, 11 Aug 1962, *Kuijt 2581* (UBC); Turrialba grounds, on *Theobroma cacao*, *Aubroma augusta*, *Davyalis hebecarpa*, and *Phoradendron piperoides*, 24 Jul 1962, *Kuijt 2498* (UBC); East of Tres Ríos, west of Los Altos, near railroad bridge, 200 yards up the creek from 1st bridge, 13 Jun 1962, *Kuijt 2455* (UBC); East of Tres Ríos, along camino on hill opposite shrine at Los Altos, on Annonaceae, 13 Jun 1962, *Kuijt 2430* (UBC). **Limón**: Pandora, on *Citrus* sp., *Theobroma cacao* and others, 31 May 1962, *Kuijt 2413* (UBC). **Puntarenas**: Golfito, on small planted tree, sea level, 2 Aug 1962, *Kuijt 2568* (UBC). **San José**: 8 miles from San Isidro del General towards Dominical, on *Coffea*, 24 June 1962, *Kuijt 2440* (UBC).

PANAMA. **Bocas del Toro**: along railroad near station at Milla 10, 27 Jul 1971, *T.B. Croat & D.M. Porter 16349* (MO); hillside above Almirante, on cacao, 28 Nov 1971, *A. Gentry 2744* (MO); Chiriquicito to 5 mi S along Río Guarumao, 5--7 Jun 1967, on *Piper*, *W.H. Lewis et al. 2110* (MO). **Canal Zone:** along Chagres River, near mouth at end of road 82, ½ km S of Fort San Lorenzo, sea level, 27 Mar 1974, *M. Nee 10882* (MO)**;** Pipeline Road between markers 7 and 11, NW of Gamboa, 4 Oct 1969, *W.H. Lewis, & R.A. Sharp 31* (MO). **Chiriquí**: Fortuna Dam region, 8°45'N, 82°15'W, 1050 m, along Quebrada Arena near continental divide, 9 Mar 1986. *G. McPherson 8748* (MO); 1.6 km W of Puerto Armuelles, along roadside and stream, 50 m, on *Jatropha curcas*, 18 Feb 1973, *T.B. Croat 21939* (MO). **Coclé:** foothills of Cerro Pilon, near El Valle, 900 m, on *Homalium*, 5 Oct 1967, *J. Duke & M. Correa 14676* (MO); La Mesa, 2200 ft, 4 Jan 1974, *J.D. Dwyer & M. Nee 11944* (MO). **Colón:** along stream 3 miles E of Transisthmian highway on road to Salamanca, 100 m, 19 Dec 1972, *A. Gentry 6729* (MO); E Santa Rita Ridge, 14 Feb. 1968, *M.D. Correa 690* (MO); hills just N of Río Guanche, 1--200 m, 16 Nov 1975, *G. Davidse & W.G. D'Arcy 10074* (MO); along Río Boqueron near No.1 manganese mine E of Salamanca, 50 m, 9.35'N 79.32'W, 3 Jul 1982, *S. Knapp, N. Hollobrook & M. Vodicka 5806* (MO). **Darien:** vicinity of Piñas, 2 Mar 1967, *J. Duke 10644* (MO); Río Balsa between Manene & Guayabo, 8 Nov 1967, *J. Duke & N. Nickerson 14946* (MO). **Panamá**, Cerro Jefe, Los Altos de Cerro Azul, Paseo Cerro Jefe just past bridge over Río Vistamares, secondary forest, on *Vismia macrophylla*, 800 m, 09°12'56.31"N, 79°24'5.28"W, 15 June 2014, *J. & L. Harrison 635* (UCH, MO); Cerro Jefe, Los Altos de Cerro Azul, Paseo Himalaya opposite lot 34 of El Frente subdivision, wet premontane forest, on *Vismia macrophylla*, 800 m, 09°12'6.76"N, 79°24'52.22"W, 15 June 2014, *J. & L. Harrison 636* (UCH, MO); Cerro Azul, Cerro Azul towers area on road to Los Altos de Cerro Azul, on leafless, unidentified tree, 585 m, 09°9'25.43" N, 79°24'59.85"W,15 June 2014, *J. & L. Harrison 637* (UCH, MO); Brushy roadside, premontane wet forest area 8.5 km by road NE of Lago Cerro Azul on road to Cerro Jefe, 800

m, 6 May 1974, *M. Nee 11472* (PMA); Cerro Jefe, cloud forest 0.4 mi from entrance to conservation area from Paseo Cerro Jefe in subdivision Los Alto de Cerro Azul, on *Vismia macrophylla*, 900 m, 09°13'34.5"N, 79°23'20.34"W, 15 Jun 2014, *J. & L. Harrison 634* (MO; UCH*)*. **San Blas**: El Llano-Cartí road, km 19.1, 350 m, 9°19'N, 78°55'W, 14 Jun 1985, *G. de Nevers & H. Herrera 5836* (MO); along canal just N of Mandinga Airport, 27 Oct 1967, *J. Duke 14852* (MO). **Veraguas:** Trail from Bajo Chitra to Río Gatú, cloud forest, Pacific slope, 8°34'N, 82°56'W, 650--750 m, 14 Jan. 1986, *G. de Nevers & G. McPherson 6783* (MO); vicinity of Santa Fé on slopes of CerroTute-Arizona above school at Alto Piedras, 8°30'N, 81°10"W, 820 m, 28 Jan 1989, *G. McPherson 13662* (MO).

The fruits of *Oryctanthus* in Panama are said to be consumed by a variety of birds, mostly residents (Leck 1972). *Vismia macrophylla* appears to be the favorite host of *O. callicarpus* on Cerro Jefe; this is possibly related to the fact that the host's fruits are also eaten by birds (Croat 1978).

Inflorescence length and flower number cannot be accurately specified in *Oryctanthus callicarpus* and most of its congeners, as flower production is essentially continuous. Inflorescences as small as 1 cm may start flowering and eventually become many times as long. In *Croat 2139* (MO), for example, one inflorescence is ca. 11 cm long and has borne some 200 flowers; it is still elongating.

Oryctanthus plants almost universally develop basal epicortical roots bearing secondary haustoria, such roots being absent from their branches. However, the host tree apparently may exert some influence over this development. In a related species in Ecuador, *O. alveolatus* (Kunth) Kuijt, it has been shown that a massive primary haustorium develops without any roots if the host is *Euphorbia latazi* Kunth (Kuijt 1989). In our scrutiny of Panamanian *O. callicarpus*, several instances have been seen where, similarly, a massive primary haustorium develops without any evidence of epicortical roots (for example, *Davidse & D'Arcy 10074* and *Lewis et al. 2110*, both MO).

The collection *Knapp et al. 5806* (MO) is recorded as being collected from a banana host plant. If true, this represents the only known instance of a banana serving as a mistletoe host in nature; an established seedling has been reported on this host by Kuijt & Mulder (1985) under greenhouse conditions. In fact, monocots generally are exceedingly rare as hosts for mistletoes (Kuijt & Mulder 1985).

Etymology: The epithet "*callicarpus*" refers to the beauty of the multicolored fruits.

ACKNOWLEDGEMENTS

We thank Dr. Kanchi Gandhi (GH) for nomenclatural advice.

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Fig. 1. *Oryctanthus callicarpus* (*J. & L. Harrison 636*).

Allopatric hybridization and introgression between *Juniperus maritima* R. P. Adams and *J. scopulorum* Sarg.: Evidence from nuclear and cpDNA and leaf terpenoids

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA, email Robert_Adams@baylor.edu

ABSTRACT

Previous studies of leaf terpenoid variation from throughout the range of *Juniperus scopulorum* found the populations in Wallowa, OR and British Columbia (northern Rocky Mountains) were differentiated from the central Rocky Mountain populations. Re-assessment of that data in concert with the leaf essential oil of *J. maritima*, suggest the 'divergent' populations at Wallowa and British Columbia are in fact hybrids and/or introgressants. New data from nrDNA (ITS), maldehy, and petN-psbM (cpDNA) confirm that allopatric hybridization is occurring at Wallowa, eastern WA, and southeastern BC into w Montana. nrDNA was found to be of less use in detecting hybrids than a single copy nuclear gene (SCN), maldehy. This appears to be due to concerted evolution in nrDNA. The uniform presence of either *J. maritima* cpDNA in western BC and WA or *J. scopulorum* cpDNA in eastern BC, WA, OR, and MT suggests allopatric introgression by air-borne pollen. Published on-line **www.phytologia.org** *Phytologia 97(1): 55-66 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus maritima, J. scopulorum, nrDNA, maldehy, petN-psbM,* leaf terpenoids, hybridization, introgression, Pleistocene refugia, recolonization, Wisconsin glaciation.

In 1983, I published an analysis of geographic variation in leaf oils of *J. scopulorum* (Adams, 1983) and noted that the samples from Puget Sound and Vancouver Island were the most distinct of all populations and that their oils were actually more similar to *J. virginiana* than *J. scopulorum* (Fig. 13, Adams, 1983). Subsequently, Adams (2007, 2014) recognized the Puget Sound and Vancouver Island (including the Strait of Georgia) plants as *J. maritima* based on the combined use of terpenoids, morphology and nrDNA sequence data. In 2011, I re-examined this terpenoid data set (Adams, 2011a), removing the *J. maritima* seaside populations from the analysis, thinking that *J. maritima* only grew in the Puget Sound maritime region. The major trend shows (Fig. 1) the leaf terpenoids of *J. scopulorum* to be relatively uniform throughout the central and southern Rocky Mountains. However, the populations in British Columbia, Wallowa, OR, and Kalispell, MT form a separate group (Fig. 1). There is also some differentiation of the other Montana populations (BM, BR, MM). Adams (1983, 2011a) postulated that the divergent populations (BC, OR, MT) arose from a Wisconsin age refugium group that re-invaded the region after the glacial retreat, bearing a terpenoid profile that differs from the typical *J. scopulorum*, Rocky Mountain terpenoid composition.

The discovery and verification of *J. maritima* growing in the Olympic Mountains as Krumholz plant at 1700m (Adams, Hunter and Fairhall, 2010), marked the first verified report of *J. maritima* growing away from its seaside (maritime) locations. Because the present seaside population sites in Puget Sound and the Strait of Georgia were glaciated during the Wisconsin (Fig. 7, Adams, Hunter and Fairhall, 2010), the authors postulated that *J. maritima* retreated to a refugium in the non-glaciated Olympic Mtns. and/or on newly exposed shore lands on the west coasts of Washington and Oregon (see Buckingham et al., 1995).

Re-analysis of the terpene data, by removing CM (found to be *J. blancoi,* introgressed by *J. scopulorum*, Adams, 2011b) and adding *J. maritima* (MA, Vancouver Island, BC) shows MB (Manning Park) related to *J. maritima* (MA, Fig. 2). There appears to be a cline from MA (*J. maritima*) to Manning Park, to the DB, WO, KM, WB, TB group (Fig. 2).

Recently, Moreno-Letelier, Mastretta-Yanes and Barraclough (2014) used six single copy nuclear (SCN) genes to study geographic variation in *Juniperus blancoi* that had been used by Li et al. (2012). An evaluation of five of these SCN genes was conducted between *J. maritima* (Brentwood Bay, BC) and *J. scopulorum* (Kamas, UT). cc13333 (515bp) gave 0 differences; chs (600 bp) had 1 difference, but was polymorphic in *J. maritima*; LHCA4 (742bp) had 3 differences, but all were polymorphic in one of the taxa; MYB (946bp) had 2 differences, but both were polymorphic in one of the taxa.

However, maldehy (522bp in *J. maritima* and 529 bp in *J. scopulorum*) had a 7 bp indel and 2 differences that separated the taxa. Maldehy is a putative malate dehydrogenase (Dvornyk, et al., 2002; Li et al., 2012).

The purpose of the present paper is to report on analyses of these northwestern populations using nrDNA (ITS), SCN (single copy nuclear) maldehy (Moreno-Letelier et al., 2014) and petN-psbM (cpDNA).

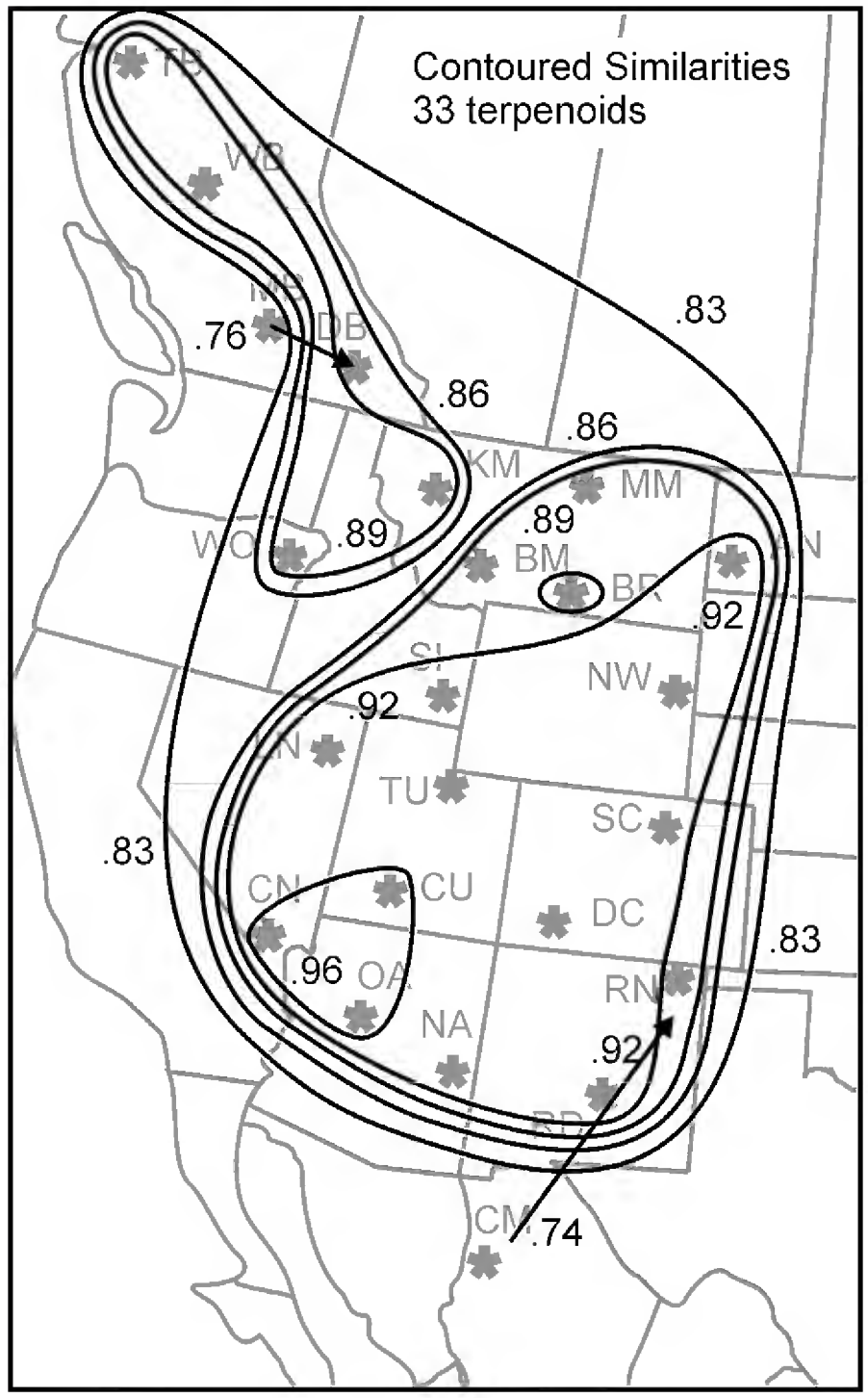

Figure 1. Contoured similarities among populations of *J. scopulorum* based on 33 terpenoids. See text for discussion. From Adams, 2011.

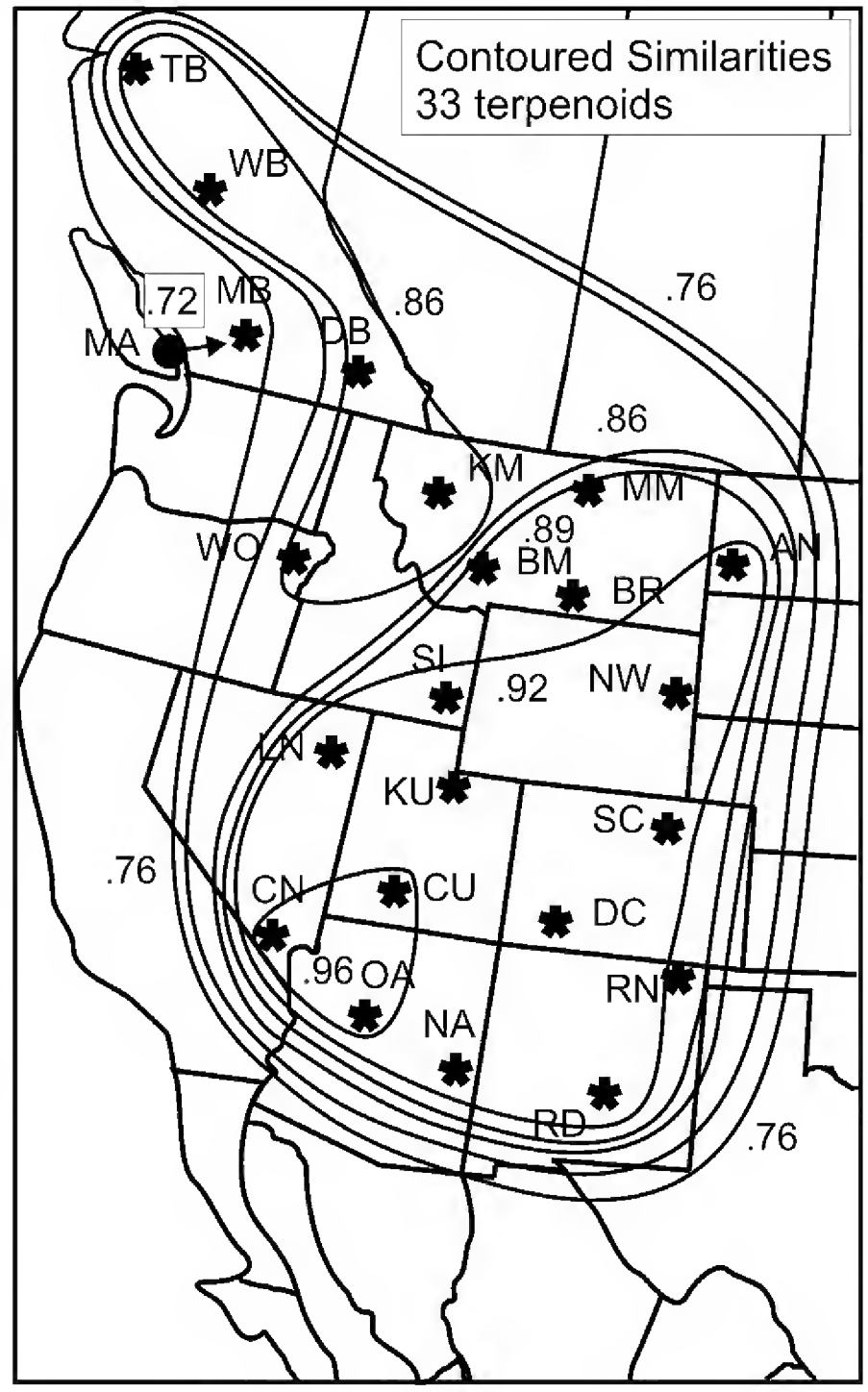

Fig. 2. Modified analysis: CM was removed and MA (*J. maritima*, Vancouver Island) was added. Notice the linkage between MA (*J. maritima*) and MB (Manning Park). Note: KU = TU in Fig. 1.

MATERIALS AND METHODS

Plant material: (species, population acronym, location, vouchers):

J. maritima: BB, Brentwood Bay, Vancouver Island, BC, *Adams 11056-11058;* CB, Cowichan Bay, Vancouver Island, BC, *Adams 11061-11063*; LI, Lesqueti Island, BC, *Adams 11064-11066*; Vancouver Island, BC; PS, San Juan Island, *Adams 11067, 11068*; Whidbey Island, *Adams 11075*; Fidalgo Island State Park, *Adams 11076*; Skagit Island, *Adams 11077-1178* (*11077* is the national big tree for *J. scopulorum*, but should be the *J. maritima*, national big tree); WL, Williams Lake, BC, *Adams 13436-13440*; Cache Creek, BC, *Adams 13431-13435*; MP, Manning Park, BC, *Adams 13426-13430*;

J. scopulorum: Reference, Kamas, UT, *Adams 10895-10899* and Glorieta Pass, NM, *Adams 10933-10935*.

Putative *J. maritima x J. scopulorum*: CR, Creston, BC, *Adams 14026-14030*; FH, Fairmont Hot Springs, BC, *Adams 13421-13425, 14001-14010*; *Adams 14001-14010*; Northport, WA, *Adams 14031-14035*; BV, Beverley, WA, *Adams 14036-14040*; WO, Wallowa Mtns., OR, *Adams 11935-11939*; KM, Kalispell, MT, *Adams 12995-12999*; Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. PCR for maldehy used: maldehyF8 5' GTGATTGGGTGCTTGGTACAC 3'; maldehyR531 5' AGTGGCATCCAGTTTTTCCTT 3', annealing temperature of 60° C, buffer E and 35 cycles.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Minimum spanning networks were using PCO3d and MINSPAN software (Adams et al., 2009; Adams, 1975; Gower, 1966, 1971; Veldman, 1967).

RESULTS AND DISCUSSION

DNA sequencing gave: nrDNA (1270bp), with 5 substitution differences between the reference populations of *J. maritima* (BB) and *J. scopulorum* (KU, GN); petN-psbM (828bp), 8 nucleotide differences plus a 7 bp indel; maldehy (522bp, *maritima*; 529 bp, *scopulorum*), 2 differences plus a 7 bp indel. Each of these sequences displayed fidelity in the reference populations (Table 1). Based on these distinct differences, an effort was made to classify each plant as to species or hybrid for maldehy and nrDNA. Of course, it may be that some positions will be heterozygous by chance or from relictual speciation.

Table 1 shows the classification of 80 individuals for each of these three gene regions. Both *J. maritima* and *J. scopulorum* are uniformly classified in their reference populations. All of the samples from the Puget Sound - Strait of Georgia - Olympic Mtns, plus Manning Park were uniformly classified

as *J. maritima*, except for *11063*, Cowichan Bay, Vancouver Island, for which maldehy was heterogenic for both substitutions, and, thus, classified as a hybrid. nrDNA was much more conserved in detecting hybrids, with only 4 hybrids compared to maldehy that found 16 hybrids (Table 1). In only one case (13421, Fairmont Hot Springs, BC) did nrDNA and maldehy classify the same tree as a hybrid. The conserved nature of the multi-copy nrDNA (up to millions of copies per cell, Liao, 1999) might be due to concerted evolution (Liao, 1999). Liao (1999) argues that because rRNAs are structural molecules, multiple gene copies are necessary to supply the demand for ribosomal subunits in the cell. Since these sub-units function only when assembled into a large complex, homogeneity of rRNAs is critical for regular, functional ribosome assembly and translation to function normally. Liao (1999) concludes that "a possible biological function of concerted evolution is to maintain homogeneous gene copies in a family so that homogeneous transcripts can be produced." However, concerted evolution is thought to be a slow process over numerous generations. Hybrids would seem likely to be heterozygous for both parents nrDNA.

The distribution of cpDNA (petN-psbM) shows a clear trend (Fig. 3) with *J. maritima* petN confined to the western BC, Vancouver Island - Puget Sound, and Olympic Mtns., with the exception of 2 trees in the Wallowa Mtns., OR (WO). Likewise, *J. scopulorum* petN is confined to southeastern BC, eastern WA, Kalispell, MT (KM) and 3 trees in the Wallowa Mtns. (Fig. 3). The pattern is suggestive of *J. scopulorum* pollen flow carrying petN towards the northwest. The four nrDNA hybrids are found in the Williams Lake (WL) and Fairmont Hot Springs (FH) populations (Fig. 4). Interestingly, no typical *J. scopulorum* nrDNA was found in the study area (Fig. 4).

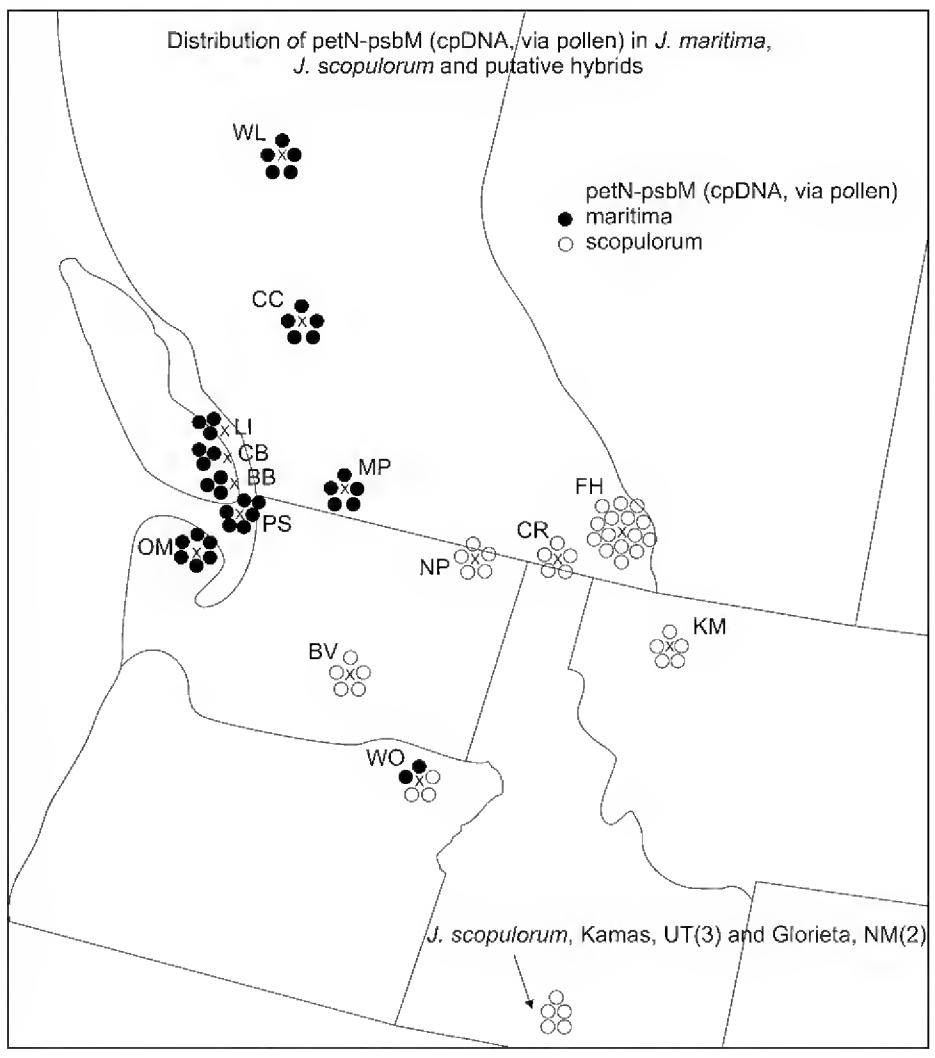

Figure 3. Classification by cpDNA (via pollen). Note the sharp break in western BC in petN.

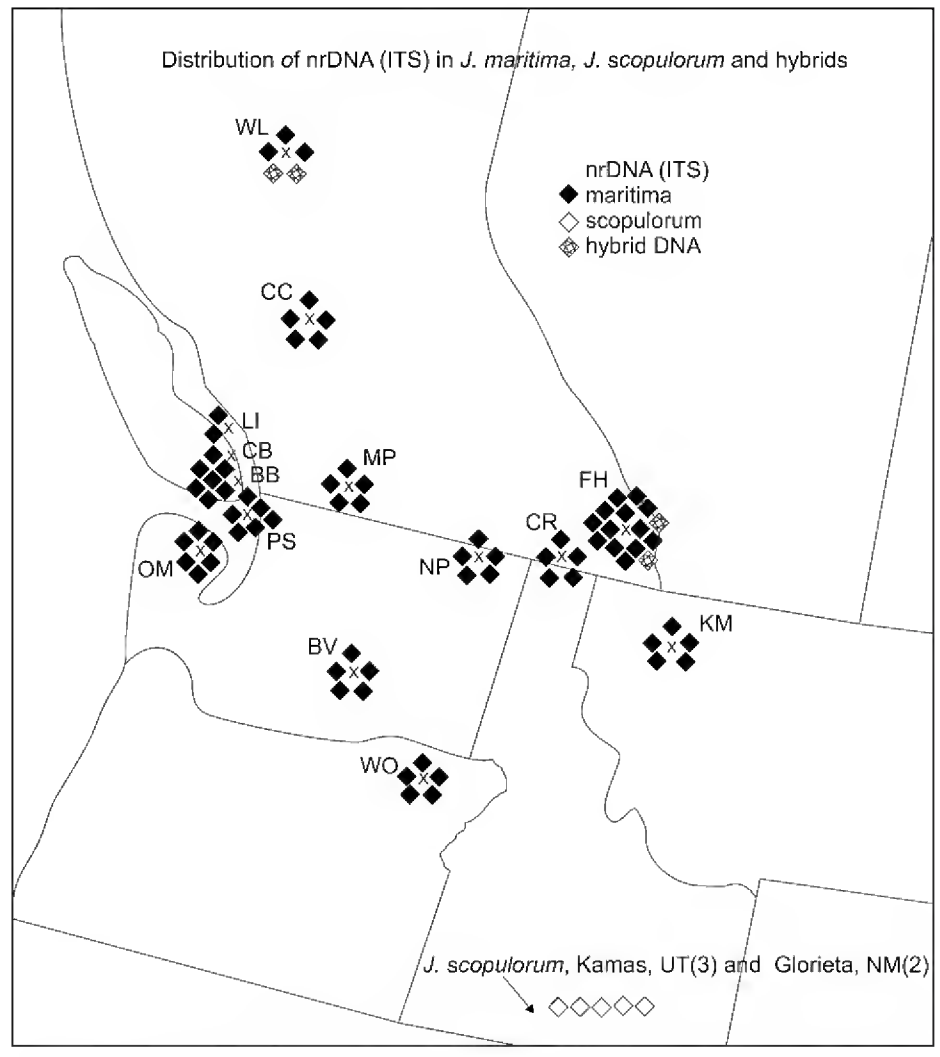

Figure 4. Classification by nrDNA. No typical *J. scopulorum*-nrDNA is present in the study area.

The distribution of maldehy types gives an interesting comparison to nrDNA and petN (Fig. 5). Again, as with nrDNA, no homogenic *J. scopulorum* maldehy trees were found. However, homogenic *J. maritima* maldehy individuals were widespread across the study area (Fig. 5). One hybrid was found in the CC (Cache Creek) population, whereas all the other hybrid maldehy plants were in eastern BC,

Beverly, WA (BV), Wallowa Mtns. (WO) and Kalispell, MT (KM). Kalispell (KM) and Wallowa (WO) are at the northwestern boundary of typical *J. scopulorum* (as judged by the terpene contour map, Figs. 1,2). This distribution is similar to that of petN (via pollen flow) that, again, suggests introgression from *J. scopulorum* via pollen.

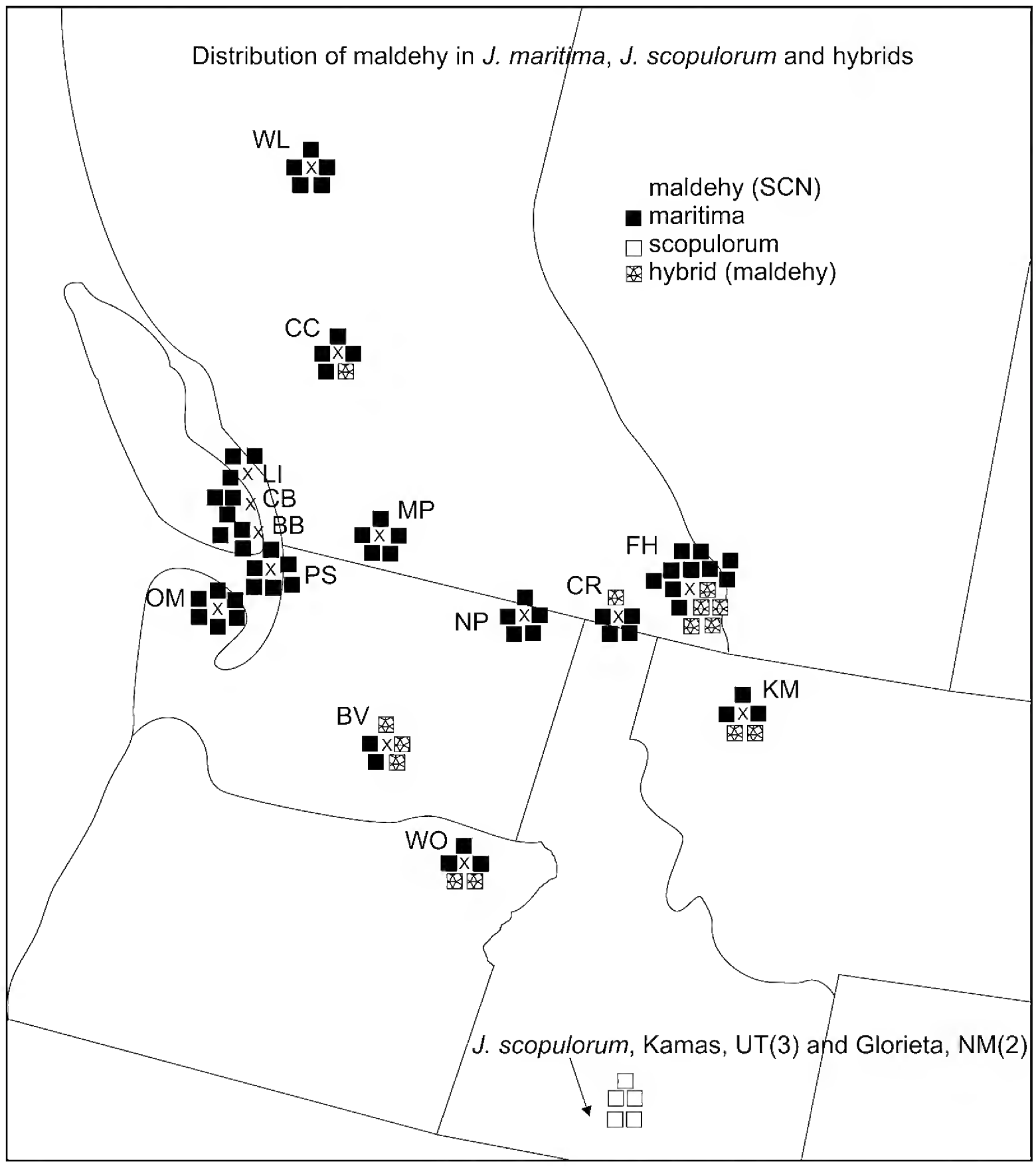

Figure 5. Distribution of *J. maritima, J. scopulorum* and hybrid as per the classification by their maldehy sequences. Notice the lack of any pure, *J. scopulorum*-maldehy individuals.

Figure 6 shows combined mapping using all three gene classifications. The area west of the dashed line appears to separate typical *J. maritima* populations from intermediate individuals (east of the dashed line). Two individuals (in the BB and CC populations) are intermediate in maldehy, along with two individuals at Williams Lake (WL) that are intermediate in nrDNA (Fig. 6). No individuals that are pure in all three genes are present east of the dashed line (central BC and WA). Wallowa (WO) is the only eastern location in which individuals (2) contained *J. maritima* cpDNA (petN). Fairmont Hot

Springs (FH) has the most hybrid individuals as well as the only individual that was classified as an hybrid in both maldehy and nrDNA (Fig. 6).

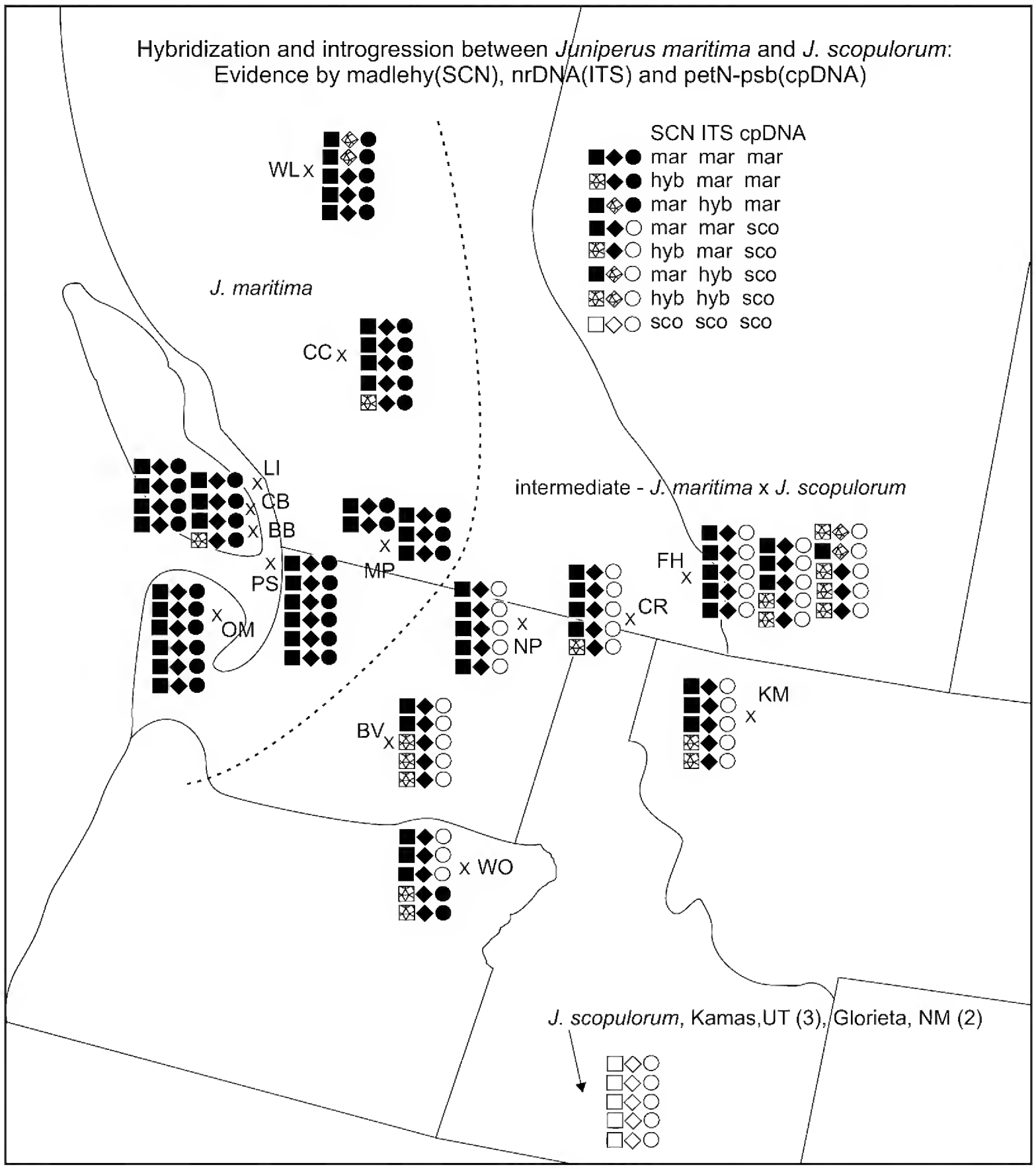

Figure 6. Combined classification based on maldehy, nrDNA, and cpDNA.

Analysis using Principal Coordinates (PCO) of the terpenoid data (Fig. 2) gives a slightly different view from the contoured similarities (Fig. 7). All the central Rocky Mtn. *J. scopulorum* populations are grouped together (shaded oval, Fig. 7). Populations WO, WL, FH, KM, and to a lesser extent, BM, are intermediate, suggesting hybridization and/or introgression. The two divergent northeastern populations (MM, BR) link to RN (Raton, NM), rather than being intermediate to *J. maritima* (Fig. 7), suggesting they are divergent, but not hybrids or introgressants with *J. maritima*. Of course, the divergence of MM and BR could reflect gene flow with *J. virginiana*. The pattern of variation presented by leaf terpenoids (Fig. 7) seems most like that of maldehy (Fig. 5).

It should be noted that although it seems intuitive that hybrids would have intermediate amounts of terpenes, Adams and Tsumura (2012) found that in *Cryptomeria japonica* hybrids, cis-thujopsene,

widdrol and cedrol were inherited in Mendelian fashion with a second (dominant/recessive) gene involved. However, several of the F_1 hybrids had oil very similar to the Haava parent's oil. In a study of the inheritance of the leaf terpenoids of *Pseudotsuga menziesii* var. *menziesii* x var. *glauca*, Adams and Stoehr (2013) found cross *menziesii* 226 x *glauca* 267 produced four hybrids with oils very similar to the glauca parent and 6 F_1 hybrids with intermediate oils. In a second cross, of the 10 major terpenoids, 8 showed dominance with values like one of the parents. Nine of the terpenes were transgressive to both parents. So it may not be unexpected that the PCO of terpenes, in the present study, show the putative hybrids' oils to be more like one of the parents (*J. scopulorum*, Fig. 7).

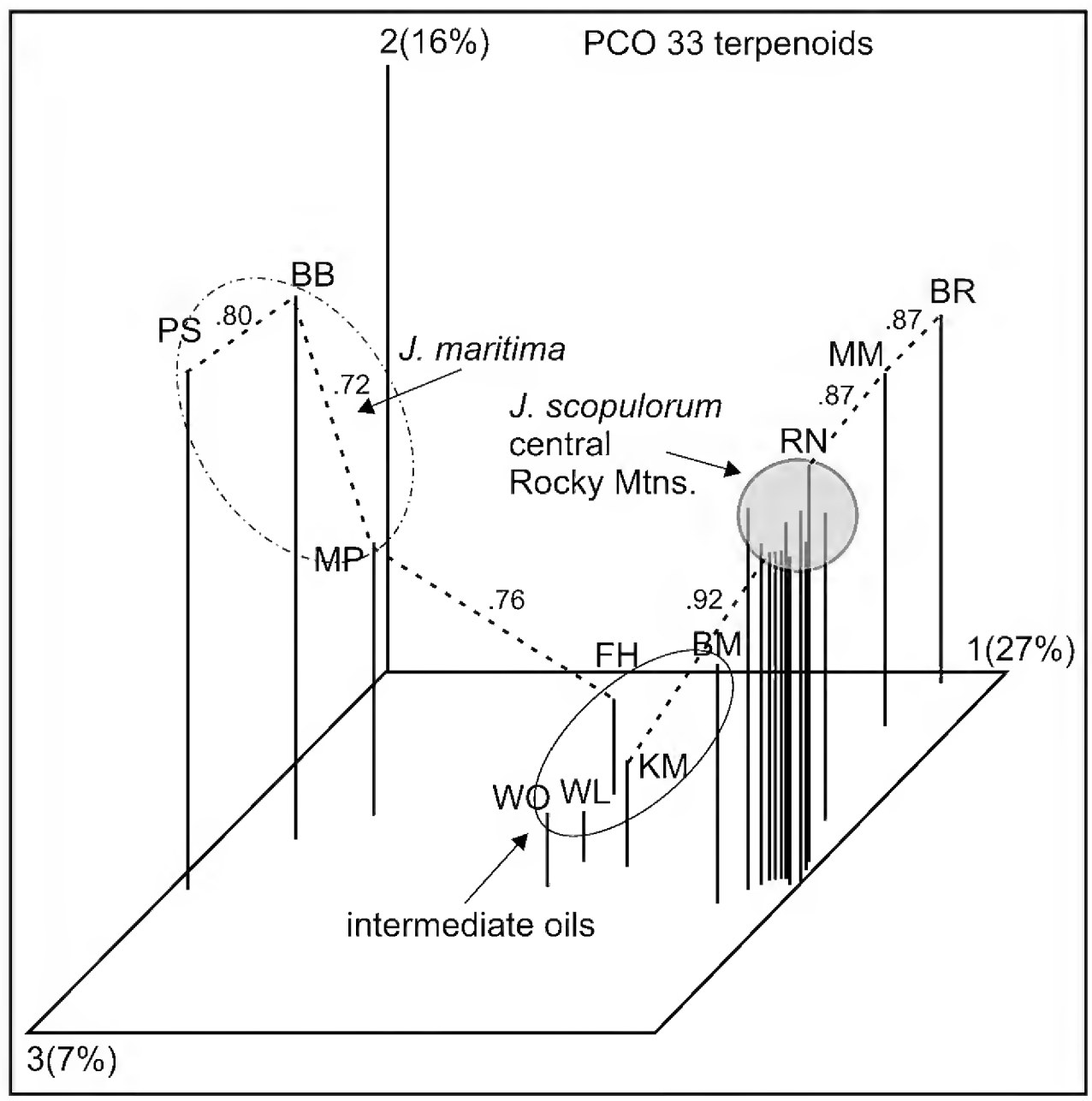

Figure 7. PCO of *J. maritima, J. scopulorum* and putative hybrid populations based on 33 leaf terpenoids. The dashed lines show the minimum spanning network between major groups. Numbers above the lines are similarities.

Pleistocene Patterns

The late Wisconsin maximum ice advance is shown in figure 8 (based on Flint, 1971 and Crandell, 1971). All of the Canadian *J. maritima* and hybrid populations were glaciated. In addition, the Kalispell (KM), Missouri River (MM) and Amidon, ND (AN) populations were probably exterminated. Other populations (BM, BR and NW) were likely displaced by boreal forests and tundra (Flint, 1971; Porter, 1971). *Juniperus scopulorum* is a lower montane species. With the widespread lowering of vegetation zones, it likely moved to lower, drier habitats throughout most of the central Rocky Mountains. Adams (1983) reviewed the literature on packrat middens and pollen profiles. Wells (1970) and Martin and Harwell (1957) suggested that life zones descended 300 to 1100 m throughout the southwest and Great Basin from 13,500 to 10,000 ybp. The current separation of *J. scopulorum* and *J. virginiana* appears to have been bridged with the eastward expansion of *J. scopulorum* and the western expansion of *J. virginiana*. Trees of *J. scopulorum* are currently growing in ravines in northeastern New

Mexico and western Oklahoma panhandle, while *J. virginiana* has now migrated westward into the Canadian River canyons in the Texas panhandle. The population of *J. scopulorum/virginiana* in Palo Duro Canyon resembles both species and is likely a relictual stand of hybrid origin (Adams, 1983).

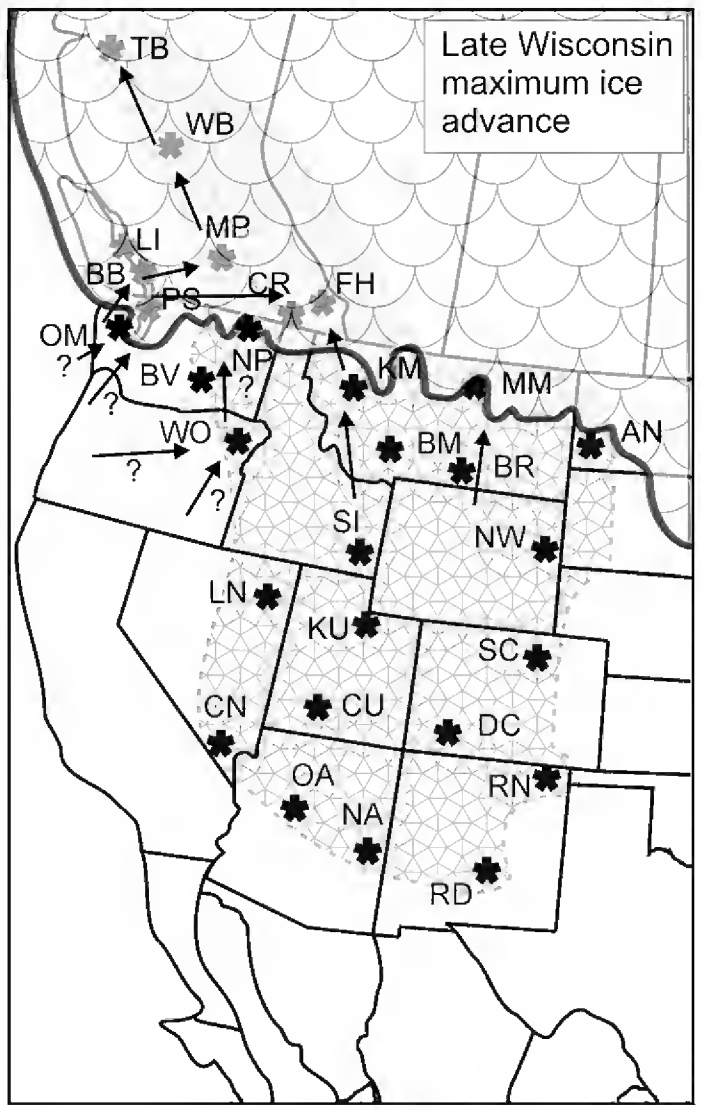

Figure 8. Putative re-colonization routes *J. maritima* and *J. scopulorum* following maximum ice advance during the late Wisconsin (ice boundary based on Flint, 1971;Crandell, 1971).

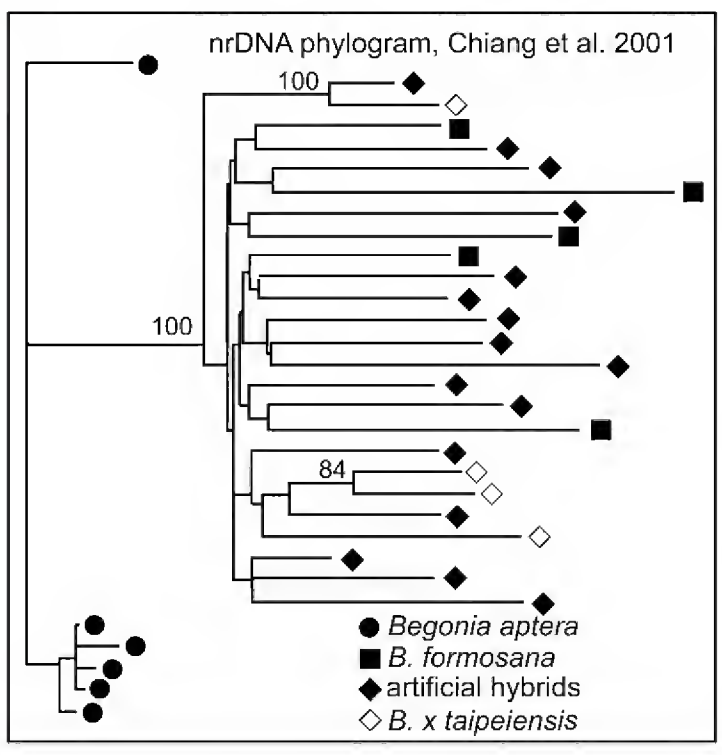

Figure 9. Phyogram based on nrDNA for *Begonia* and hybrids (adapted from Chiang, et al. 2001).

With the retreat of the Wisconsin glacial ice, and the subsequent altithermal period 9000 to 5000 ybp (Wells, 1970), *Juniperus* expanded into the drying, higher elevation habitats that it occupies today. Figure 8 shows the proposed post-Pleistocene re-colonization of the northern portion of the ranges of *J. maritima* and *J. scopulorum*. The *J. maritima* BC populations could have been recolonized by seed from a Wallowa Mtns. refugium (WA, Fig. 8) and thence northward to the present day northern-most population at Telkwa, BC (TB). At Telkwa, *J. scopulorum* is found on dry, southeast facing slopes (ca. 45° - 60°). It seems likely that *J. maritima* that grows along the seashore in western BC and Puget Sound, WA was re-colonized from a refugium south of the Olympic Mtns. or western WA/ Oregon.

Of course, the Wallowa population may have been displaced lower, and perhaps a bit to the south during the Wisconsin. The Amidon, ND (AN) population is similar to populations in the central Rocky Mountains and seems likely to have been derived by seed from the nearest *J. scopulorum* population (perhaps near Newcastle, WY, NW) or any of the scarp-land *J. scopulorum* populations to the south.

It may be that heterozygous trees are the result of previous hybridization(s) or a relict from speciation. Or, the nrDNA may reflect concerted evolution in homogenizing individuals. However, Chaing et al. (2001) found that in the artificial hybrids between *Begonia aptera* (pollen) and *B. formosana* (ovule), nrDNA was predominantly that of the maternal parent, *B. formosana* (Fig. 9). Volkov, et al. (1999) reported that one of the parental nrDNAs was eliminated the allopolyploid genome of cultivated tobacco. Fukuoka et al. (1994) found that the nrDNA in γ-ray irradiated tetraploid rice was homogenized in a short time.

Aguilar et al. (1999) made artificial hybrids between *Armeria villosa* ssp. *longiaristata* and *A. colorata;* then examined the inheritance of nrDNA in F_1 and F_2 generations. They found the expected additive pattern in polymorphisms for five of the six variable sites in F_1 plants. However, in the F_2 generation, there was a bias towards one parent (*A. colorata*). Backcrosses showed homogenization of five of the polymorphic sites to the recurrent parent.

Okuyama et al. (2005) examined introgression in *Mitella* using nrDNA ITS and ETS, and cpDNA and found

that cpDNAs revealed the most introgression, ITS regions showed a moderate amount and the ETS region gave no evidence of introgression. They concluded that non-uniform concerted evolution between the ETS region and ITS regions may explain these different patterns of introgression.

Additional studies are needed to resolve this and to determine the extent of introgression of *J. maritima* genes into the range of *J. scopulorum* in Montana and adjacent areas (research in progress).

ACKNOWLEDGEMENTS

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Table 1. Classification of 80 *Juniperus* individuals based on maldehy (SCN), nrDNA and petN-psbM (cpDNA).

Samples (trees)	maldehy	nrDNA	petN/psbM
10895 scopulorum, Kamas, UT	scop	scop	scop
10896 scopulorum, Kamas, UT	scop	scop	scop
10897 scopulorum, Kamas, UT	scop	scop	scop
10933 scopulorum, Glorietta, NM	scop	scop	scop
10934 scopulorum, Glorietta, NM	scop	scop	scop
11056 maritima, Brentwood Bay, VI	marit	marit	marit
11057 maritima, Brentwood Bay, VI	marit	marit	marit
11058 maritima, Brentwood Bay, VI	marit	marit	marit
11061 maritima, Cowichan Bay, VI	marit	marit	marit
11062 maritima, Cowichan Bay, VI	marit	marit	marit
11063 maritima, Cowichan Bay, VI	**hybrid**	marit	marit
11999 maritima, Olympic Mtns., 912m,	marit	marit	marit
12000 maritima, Olympic Mtns., 912m,	marit	marit	marit
12001 maritima, Olympic Mtns., 912m,	marit	marit	marit
12002 maritima, Olympic Mtns., 1671m,	marit	marit	marit
12003 maritima, Olympic Mtns., 1671m,	marit	marit	marit
12004 maritima, Olympic Mtns., 1671m,	marit	marit	marit
11064 maritima, Yellow Point Lodge, VI	marit	marit	marit
11065 maritima, Lesqueti Island, BC	marit	marit	marit
11066 maritima, Lesqueti Island, BC	marit	marit	marit
11067 maritima, Friday Harbor, San Juan	marit	marit	marit
11068 maritima, English Camp, San Juan	marit	marit	marit
11075 maritima, sand dune, Whidbey Isl.	marit	marit	marit
11076 maritima, Fidalgo Isl. St. Pk	marit	marit	marit
11077 maritima, Skagit Isl. ca 360 yr old	marit	marit	marit
11078 maritima, Skagit Isl., WA	marit	marit	marit
13426 maritima, Manning Park, BC	marit	marit	marit
13427 maritima, Manning Park, BC	marit	marit	marit
13428 maritima, Manning Park, BC	marit	marit	marit
13429 maritima, Manning Park, BC	marit	marit	marit
13430 maritima, Manning Park, BC	marit	marit	marit
13431 Cache Ck, BC	marit	marit	marit
13432 Cache Ck, BC	marit	marit	marit
13433 Cache Ck, BC	**hybrid**	marit	marit
13434 Cache Ck, BC	marit	marit	marit
13435 Cache Ck, BC	marit	marit	marit
13436 Williams Lake, BC	marit	**hybrid**	marit
13437 Williams Lake, BC	marit	marit	marit
13438 Williams Lake, BC	marit	**hybrid**	marit
13439 Williams Lake, BC	marit	marit	marit
13440 Williams Lake, BC	marit	marit	marit
13421 Fairmont Hot Sprs, BC	**hybrid**	**hybrid**	**scop**
13422 Fairmont Hot Sprs, BC	marit	marit	scop
13423 Fairmont Hot Sprs, BC	marit	marit	scop
13424 Fairmont Hot Sprs, BC	marit	marit	scop
13425 Fairmont Hot Sprs, BC	marit	marit	scop
14001 Fairmont Hot Sprs, BC	marit	**hybrid**	scop

Samples (trees)	maldehy	nrDNA	petN/psbM
14002 Fairmont Hot Sprs, BC	marit	marit	scop
14003 Fairmont Hot Sprs, BC	marit	marit	scop
14004 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14005 Fairmont Hot Sprs, BC	marit	marit	scop
14006 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14007 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14008 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14009 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14010 Fairmont Hot Sprs, BC	marit	marit	scop
14026 Creston, BC	marit	marit	scop
14027 Creston, BC	marit	marit	scop
14028 Creston, BC	**hybrid**	marit	scop
14029 Creston, BC	marit	marit	scop
14030 Creston, BC	marit	marit	scop
14031 Northport, WA	marit	marit	scop
14032 Northport, WA	marit	marit	scop
14033 Northport, WA	marit	marit	scop
14034 Northport, WA	marit	marit	scop
14035 Northport, WA	marit	marit	scop
14036 Beverly, WA	**hybrid**	marit	scop
14037 Beverly, WA	marit	marit	scop
14038 Beverly, WA	marit	marit	scop
14039 Beverly, WA	**hybrid**	marit	scop
14040 Beverly, WA	**hybrid**	marit	scop
12995 Kalispell, MT	marit	marit	scop
12996 Kalispell, MT	marit	marit	scop
12997 Kalispell, MT	**hybrid**	marit	scop
12998 Kalispell, MT	**hybrid**	marit	scop
12999 Kalispell, MT	marit	marit	scop
11935 Wallowa Mtns, OR	**hybrid**	marit	scop
11936 Wallowa Mtns, OR	**hybrid**	marit	scop
11937 Wallowa Mtns, OR	marit	marit	scop
11938 Wallowa Mtns, OR	marit	marit	scop
11939 Wallowa Mtns, OR	marit	marit	scop

A molecular re-examination of phylogenetic relationships among *Juniperus*, *Cupressus*, and the *Hesperocyparis-Callitropsis-Xanthocyparis* clades of Cupressaceae

Randall G. Terry
Biology Department, Lamar University, P. O. Box 10037, Beaumont, TX, 77710, USA
rgterry@lamar.edu

and

Robert P. Adams
Baylor University, Biology Department, One Bear Place, 97388, Waco, TX 76798, USA

ABSTRACT

Previous molecular phylogenetic studies have recovered conflicting hypotheses of relationship among *Juniperus* (J), *Cupressus* (C), and *Hesperocyparis-Callitropsis-Xanthocyparis* (HCX). Conflict between nuclear genes, chloroplast genes, and nuclear and chloroplast data have all been realized in recovering all possible topologies among the three clades. In this study, we use 2.2 kb of aligned sequence from two nuclear loci, and 11.4 kb of sequence from 11 chloroplast regions, in re-examining relationships among J-C-HCX. Unlike previous studies, we find unambiguous support for relationships in the nuclear data, whether the genes are analyzed individually or in combination. In contrast, character conflict between different chloroplast partitions, or even between characters from a single region, results in nearly equally well-supported but conflicting hypotheses of relationship. Statistical tests of likelihood values indicate the chloroplast data always fails to distinguish between two of three competing sister group relationships, and in one instance cannot differentiate between any of the three possible J-C-HCX topologies. Results presented here suggest a complex evolutionary history in which molecular processes in addition to possible ancient hybridization have obscured J-C-HCX relationships. Published on-line **www.phytologia.org** *Phytologia 97(1): 67-75 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: chloroplast DNA, nrDNA, *Juniperus*, *Hesperocyparis-Callitropsis-Xanthocyparis* (HCX), parsimony, Bayesian analysis

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Cupressaceae is the third largest gymnosperm family with over 130 species in about 33 genera (Farjon, 2005; Farjon et al., 2002; Adams et al., 2009). The family is well represented in both the northern and southern hemispheres, with members occupying all habitable continents and occurring in a variety of habitats (Farjon, 2005; Adams, 2014.) Rarity and high degrees of endemism are disproportionately represented in the family, with 18 genera being monotypic and 27 having five or fewer species (Farjon, 2005). Among the more diverse genera in the family are *Juniperus* (67 species, 34 varieties), many species of which are adapted to semi-arid habitats in the northern Hemisphere, *Cupressus*, a genus of 12 species (Little, 2005) geographically centered in Asia (Mao et al., 2010) and generally known as the "Old World cypresses" (OWC), and *Hesperocyparis*, a recently recognized genus of 17 species (Adams et al., 2009; Adams et al., 2014; Wolf, 1948) from the western United States, Mexico, and central America (i. e., the New World cypresses or NWC).

A spate of phylogenetic studies published over the last decade have resulted in new perspectives on the phylogeny of *Juniperus*, *Cupressus, Hesperocyparis* and related taxa (Little et al., 2004; Little, 2005; Little, 2006; Adams et al., 2009; Yang et al., 2012; Terry et al., 2012). The recovery and taxonomic recognition of *Hesperocyparis* as distinct from *Cupressus* (Adams et al., 2009), strong support for inclusion of *Callitropsis* and *Xanthocyparis* in a lineage with *Hesperocyparis* (i. e., the HCX lineage of Terry et al., 2012; Little et al., 2004; Little, 2006; Adams et al., 2009), and studies elucidating species

relationships (Terry et al., 2012) and the recognition of new species (Adams et al., 2014) within *Hesperocyparis* and *Juniperus* (Mao et al., 2010), collectively represent our improved understanding of evolutionary and taxonomic relationships in the group.

Despite these advances, a number of outstanding questions remain. Among these are relationships among certain genera of Cupressaceae (Gadek et al., 2000; Yang et al., 2012), including those among *Juniperus* (J), *Cupressus* (C), and HCX. One of the first studies to address relationships among *Juniperus* and Old and New world representatives of *Cupressus* was that of Gadek et al. (2000), which used molecular and morphological data in addressing relationships among the major lineages of Cupressaceae. Parsimony analysis of cpDNA sequences recovered a clade containing distinct Old and New World *Cupressus* as sister to *Juniperus,* i. e., J (OWC, NWC) (Gadek et al., 2000). Two subsequent studies used cpDNA sequences to corroborate the findings of Gadek et al. (2000) in recovering a J(C,HCX) topology (Adams et al., 2009; Yang et al., 2012), while relationships among the three lineages were unresolved in a third study that used cpDNA (see Little, 2006). Three studies have used DNA sequences from a total of five nuclear loci in addressing relationships among J, C, and HCX. Two general patterns emerge from these studies: nrITS sequences always yield a C(J,HCX) topology (Adams et al., 2009; Little, 2006), and the other data sets, either alone or in various combinations, yield a HCX(J,C) topology (Little, 2006; Adams et al., 2009; Yang et al., 2012). Collectively, these findings indicate conflict between nrITS and other nuclear data sets (ABI3, 4CL, Needly, and Leafy) in resolving relationships among J-C-HCX, but in no instance are phylogenies derived from nuclear data congruent with those based on cpDNA, a finding some authors attribute to ancient hybridization (Yang et al., 2012).

In this study, we re-examine relationships among J-C-HCX using nearly 13.7 kb of aligned DNA sequence from both the chloroplast and nuclear genomes. Results from separate analyses of the cytoplasmic and nuclear data as well as combined analyses are used to re-assess relationships among J-C-HCX.

MATERIALS AND METHODS

Specimens used in this study with voucher information and GenBank accession numbers are provided in Table 1. For all specimens, one gram (fresh weight) of foliage was placed in 20g of activated silica in the field, and subsequently stored at -20°C in the lab.

DNA extraction, PCR amplifications, and preparation of sequencing templates are according to Terry et al. (2012). Briefly, total genomic DNA was extracted from 0.020 g of silica dried leaf tissue using a DNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). The psbD-trnT intergenic spacer and the trnC-trnD intergenic region containing spacer sequence and a portion of the psbM coding region were amplified and sequenced for two species of *Calocedrus*, four species of *Juniperus*, and three species of *Cupressus* (Table 1). All other sequences were previously published (Gadek et al., 2000; Little et al., 2004; Little, 2006; Terry et al., 2012) and are available in GenBank. Thermal cycling protocols for all amplifications were as follows: 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 2 min at the optimized annealing temperature, and 72°C for 2 min, followed by 72°C for 7 min. Annealing temperatures were 47.5°C for psbD-trnT and 50°C for trnC-trnD. Primer sequences and other amplification details are given in Terry et al. (2012). PCR products were purified by agarose gel electrophoresis according to Terry et al. (2012) and sequenced at McLab Inc. (San Francisco, CA).

Combining data from this study with chloroplast and nuclear sequences from GenBank produced 13.7 kbp of aligned sequence from 9 noncoding chloroplast regions (8 intergenic spacers and one intron), 2 chloroplast genes (rbcL and psbB), and 2 nuclear genes (nrITS and NEEDLY intron 2). For sequences published here, raw sequence from forward and reverse strands was assembled and aligned using Clustal

Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) or MAFFT. Computer generated alignments were manually refined using Seq-Al v.2.0a9 (Rambaut 2002). Both parsimony and Bayesian analyses were performed on each of three data sets: chloroplast data only, nuclear data only, and combined chloroplast and nuclear data. Parsimony analyses were conducted using PAUP*v.4.0b10 (Swofford 2002), with the heuristic search option in effect, simple stepwise addition of taxa, and TBR branch swapping, saving multiple trees. Branch support was assessed by conducting 1000 replicates of bootstrapping with the settings described above. Bayesian analyses were conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003) according to Terry et al. (2012). Best-fit evolutionary models were estimated for individual gene regions using the Akaike information criterion (AIC) implemented in jModelTest v.0.0.1 (Posada 2008; Guindon and Gascuel 2003) using the default settings for likelihood calculations and the uncorrected AIC. Bayesian analyses were fully partitioned by gene region, with two independent runs of four Metropolis coupled chains each. Chains were generated from different random trees and run for 1 million generations, sampling every 1,000th generation. In each run, three chains were heated using a temperature of 0.2 with one swap between chains every generation. The burnin fraction was enforced to 0.2 using the ‘‘relburnin’’ command, resulting in the first 200 of 1,000 trees being discarded, and the remaining trees pooled to construct the posterior distribution of the phylogeny. A 50 % majority-rule consensus tree was produced using the ‘‘contype = halfcompat’’ command. Convergence and mixing were assessed by examining plots of likelihood against chain generation over the course of the run and by monitoring the standard deviation of split frequencies among runs in MrBayes.

We statistically compared log likelihood values in assessing the relative support of the nuclear and chloroplast data for each the three possible J-C-HCX topologies. Three tests were performed; a 1-sided Kishino-Hasegawa (KH; Goldman et al., 2000), the Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa, 1999), and the expected likelihood weights (ELW; Strimmer and Rambaut 2002). Each test was performed on each of three user defined trees, the two trees from parsimony analysis of the cpDNA only [(HCX(J,C) and J(C,HCX)], and the single tree from the combined parsimony analysis (C(J,HCX). Maximum likelihood analyses and statistical tests of fit were performed in Tree Puzzle 5.2 (Schmidt et al., 2002). Default settings were used in all tests except a gamma distribution with four rate categories was used in estimating rate heterogeneity.

RESULTS AND DISCUSSION

Parsimony analysis of the chloroplast data only produced two shortest-length tree of 759 steps (CI=0.90, RI=0.91; Fig. 1). Bootstrapping of these data produced strong support for most branches, but relationships among J, C, and HCX were unresolved in the 50% majority rule tree (Fig. 1). Of the two most parsimonious trees recovered, one was consistent with previous reports (Gadek et al., 2000; Adams et al., 2009; Yang et al., 2012) in recovering J(C,HCX), while the other recovered HCX(J,C). Bayesian analysis of the chloroplast data also recovered a HCX(J,C) topology, but while nearly all branches had posterior probabilities (pp) of 1.0, the J-C clade was weakly supported (pp= 0.62; Fig. 2) .

In contrast to the chloroplast data, analyses of the nuclear data alone, or of combined nuclear and chloroplast data, consistently produced strong support for a clade containing J and HCX (Figs. 3-5). In addition, parsimony analysis of the nuclear data alone recovered four shortest length trees, all of which contained C(J,HCX), and strong support for a J-HCX sister group relationship (Fig 3). Similarly, parsimony analysis of the combined data produced a single tree of 1522 steps (CI=0.87, RI=0.90) in which a well-supported J-HCX clade was recovered (Fig. 5). Bayesian analysis of nuclear data alone or of combined nuclear and chloroplast data always recovered a C(J,HCX) topology with strong support (pp=1.0) for the J-HCX clade (Figs. 4 and 5).

Maximum likelihood analysis using Tree Puzzle found the HCX(J,C) and J(C,HCX) topologies explained the cpDNA nearly equally well, while the C(J,HCX) topology produced the least likely

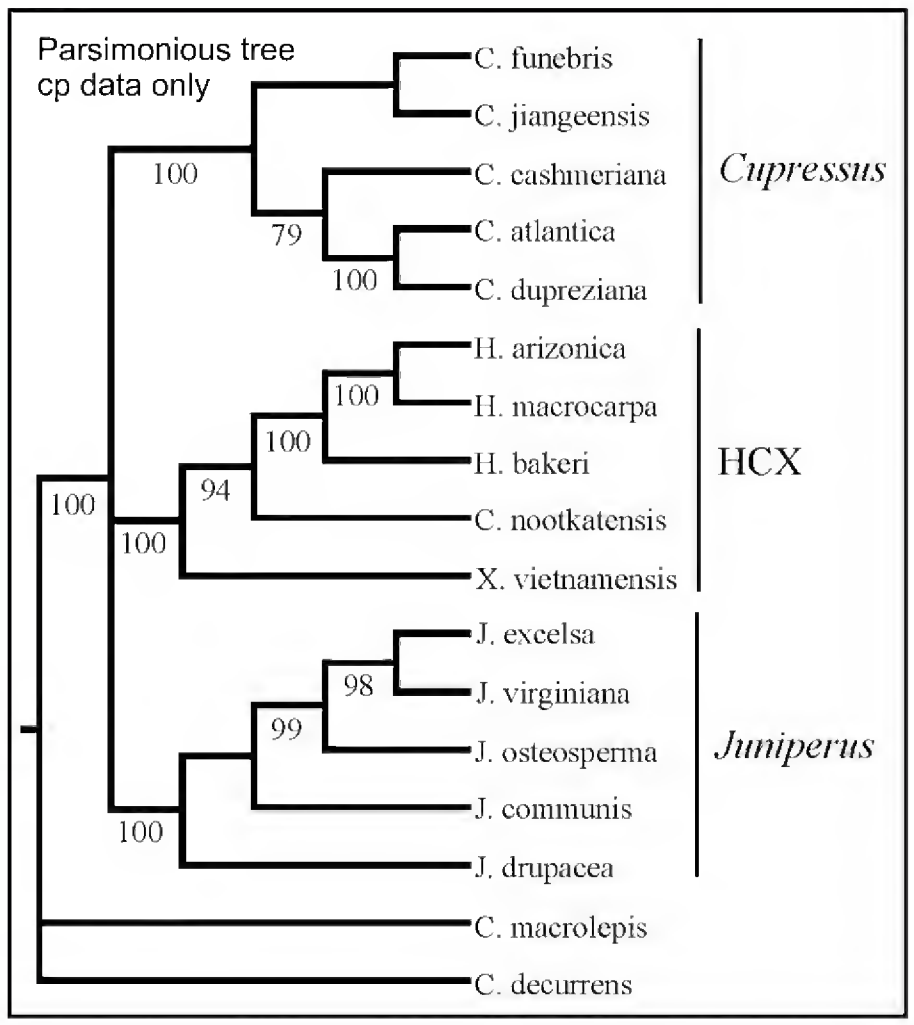


Figure 1. 50% majority-rule consensus of two most parsimonious trees generated from analysis of chloroplast data only. Length = 759 steps, CI = 0.90, RI = 0.91. Numbers below branches are bootstrap values, and are not given for values less than 50%.

explanation of the data. All tests found no significant difference between the HCX(J,C) and J(C,HCX) topologies, and one of three tests (SH) found no difference among the three possible J-C-HCX alternatives (Table 2).

Here, we re-examine relationships between *Juniperus*, *Cupressus*, and HCX with 13.7 kb of aligned DNA sequence. Our data include 2329 bp of aligned sequence from two nuclear genes (nrITS and Needly), and 11402 bp of sequence from 11 chloroplast regions (Table 1). We consistently recover a C(J,HCX) topology from the nrITS and Needly data sets, analyzed either alone or in combination. This finding is supported by results from only one previous study (i.e. Adams et al., 2009, which used combined nuclear and chloroplast data), and is in conflict with the HCX(J,C) topology recovered from analyses of several other nuclear loci

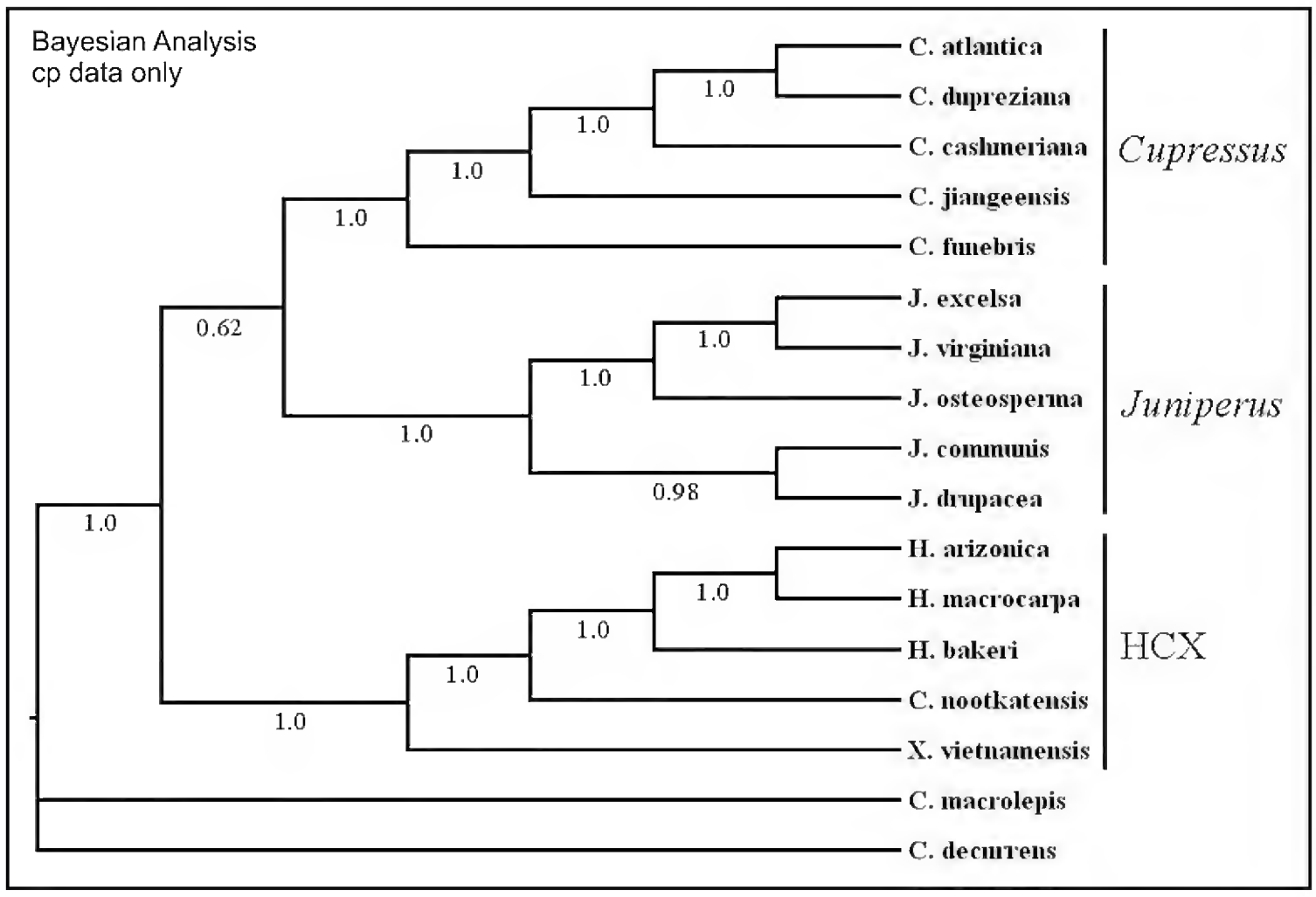
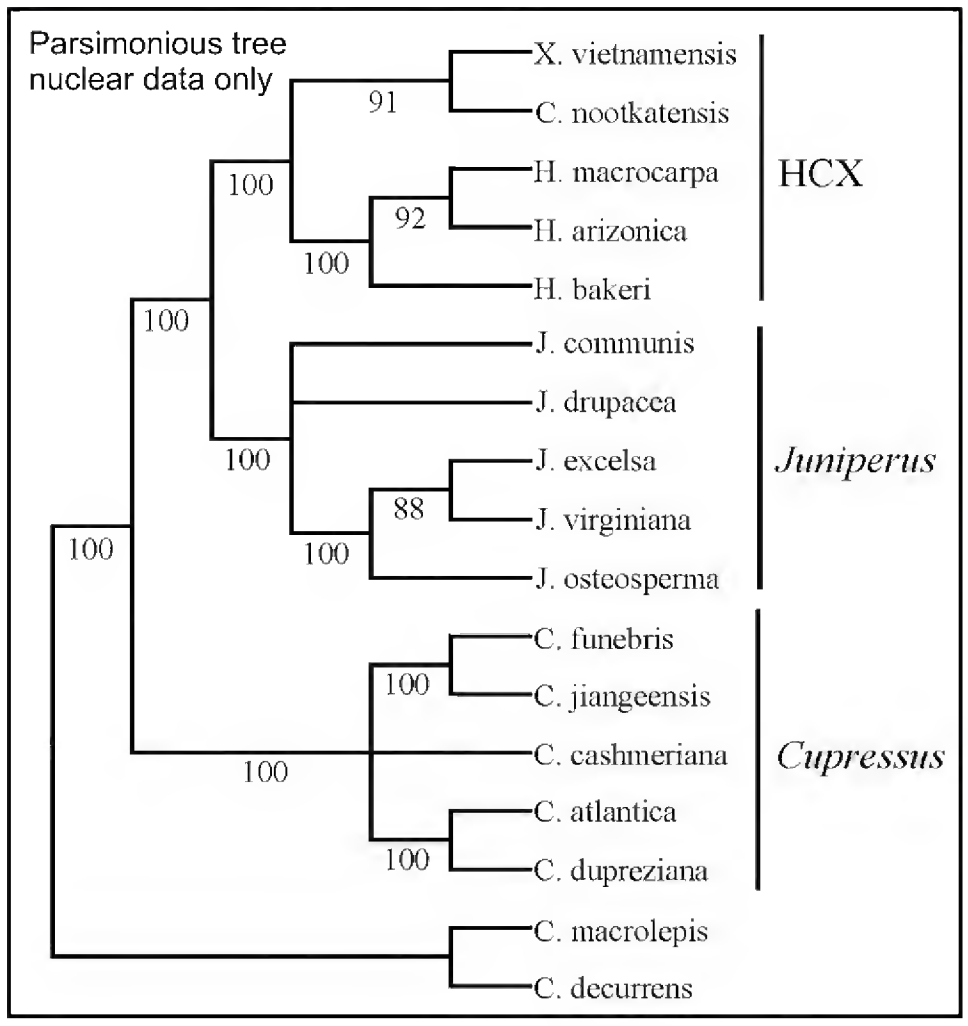


Figure 2. 50% majority-rule consensus tree generated from Bayesian analysis of chloroplast data only. Numbers below branches are posterior probabilities.



(Little, 2006; Adams et al., 2009; Yang et al., 2012). Nevertheless, we find strong support for a J-HCX sister group relationship (bootstrap=100, pp=1.0) in all analyses including nuclear data (Figs. 3-5), and note that is no instance is nuclear data unable to statistically distinguish C(J,HCX) from either of the other two J-C-HCX alternatives (data not shown). Moreover, character analysis identified 42 synapomorphies for the J-HCX clade in the combined analysis, 35 from the nuclear genes and nearly equally divided between nrITS and Needly (combined CI of 0.88), and 7 from the chloroplast data (CI=0.79).

In contrast to the nuclear data, chloroplast sequences do not provide strong support for any particular hypothesis of J-C-HCX relationship. Perhaps this is best exemplified by results in which the chloroplast data never

Figure 3. 50% majority-rule consensus of four most parsimonious trees generated from analysis of nuclear data only. Length = 754 steps, CI = 0.86, RI = 0.89. Numbers below branches are bootstrap values.

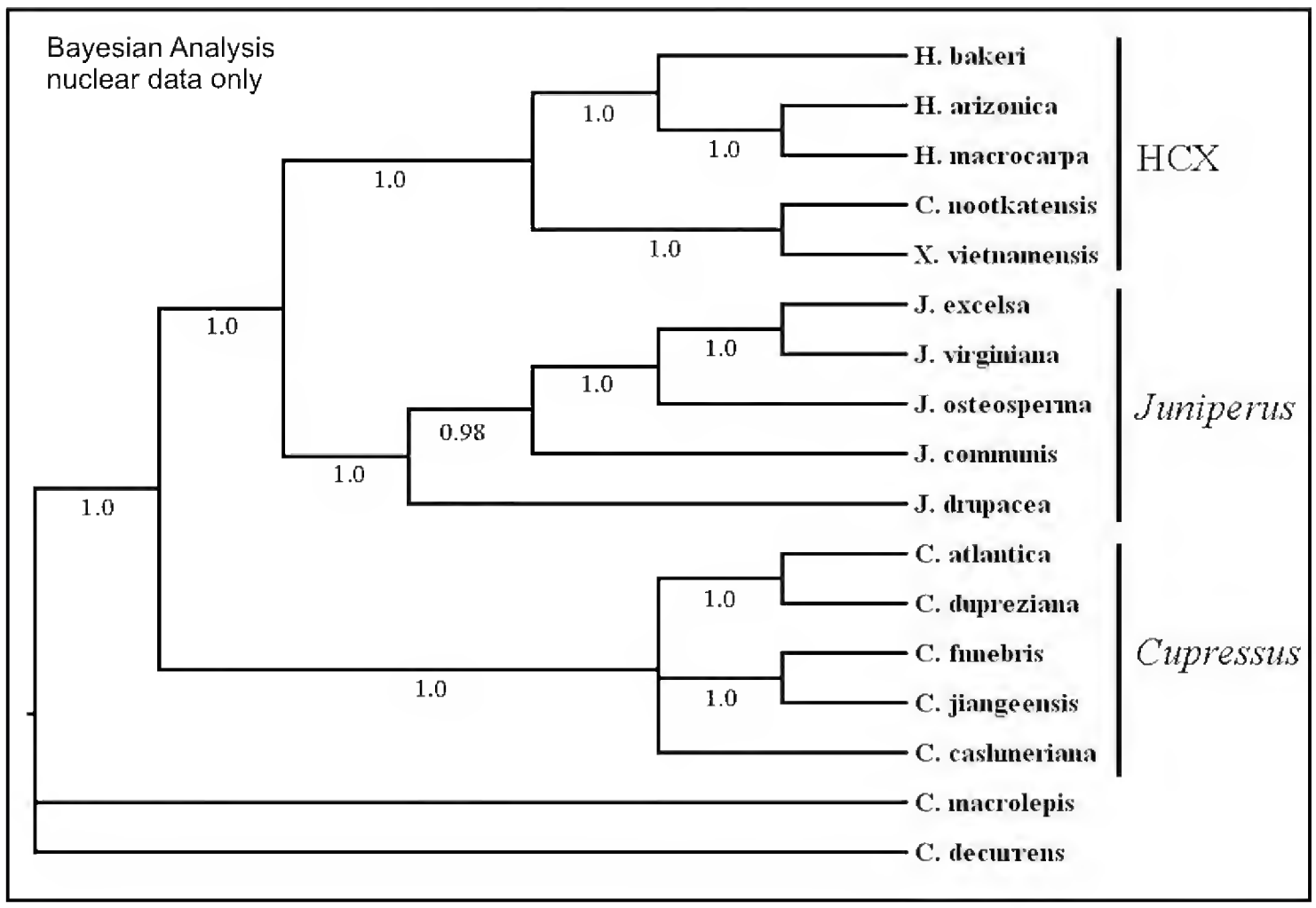


Figure 4. 50% majority-rule consensus tree generated from Bayesian analysis of nuclear data only. Numbers below branches are posterior probabilities.

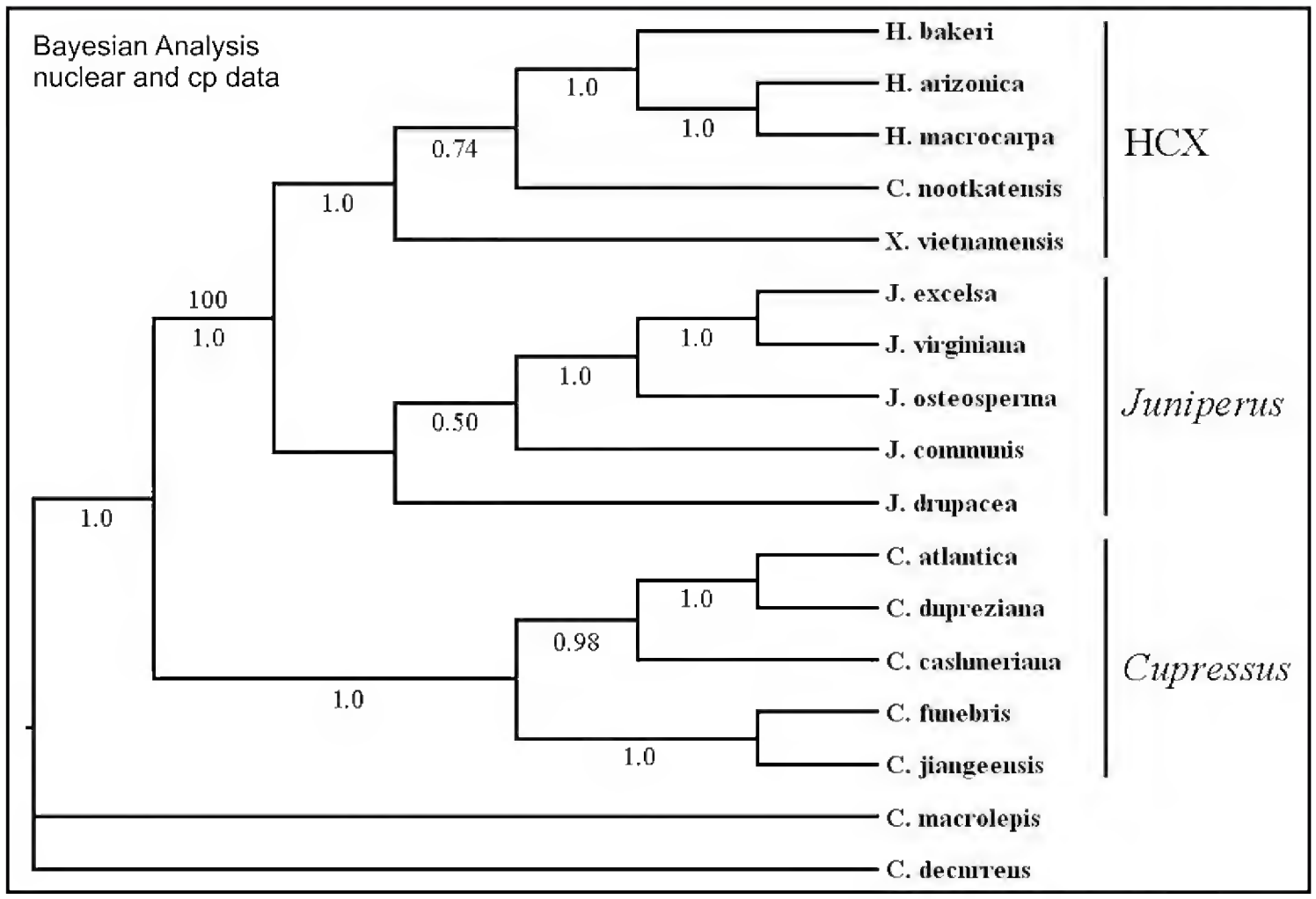


Figure 5. 50% majority-rule consensus tree generated from Bayesian analysis of all data. Numbers below branches are posterior probabilities. Parsimony analysis of all data produced one tree (1522 steps, CI=0.87, RI=0.90) having the same topology with respect to J-C-HCX relationships as the Bayesian consensus tree. The bootstrap value from parsimony analyses for the J-HCX clade is given above the branch.

distinguish between J(C,HCX) and HCX(J,C), and for one test, cannot distinguish between any of the three possible J-C-HCX alternatives (Table 2). Neither bootstrap values (data not shown) nor posterior probabilities (Fig. 2) provide strong support for sister group relationships, and in the case of parsimony analyses, the number of synapomorphies and their consistency is nearly identical for conflicting sister group hypotheses (data not shown). Previous studies based on cpDNA were either unresolved with respect to J-C-HCX relationship (Little, 2006) or have found J(C,HCX) (Adams et al., 2009; Yang et al., 2012), but in no instance has a well-supported C-HCX sister group relationship been recovered.

Some authors have suggested *Cupressus* originated through hybridization between *Juniperus* and the common ancestor of HCX, an assertion based on conflict between different nuclear loci (Needly and Leafy vs. nrITS), similarity in *Cupressus* Needly and Leafy sequences to those of both *Juniperus* and HCX, and conflict between topologies derived from cpDNA (matK) and nuclear sequences (Yang et al., 2012). Results present here are different from those of previous studies in that we find little or no conflict among different nuclear partitions in recovering a well-supported C(J,HCX). In addition, we find little support for J-C-HCX relationships in the cpDNA data, although the cpDNA data never supports a J-HCX clade, and C(J,HCX) is excluded from the other two alternatives in two of three statistical comparisons of topology (Table 2). Collectively, these findings suggest that if conflict in topologies supported by nuclear and cpDNA data is attributable to ancient hybridization (Yang et al., 2012), then other processes producing ambiguity in the chloroplast data, or conflict between different nuclear genes (Yang et al., 2012), have also been important in the evolutionary history of the group.

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		Locus												
Taxon	Voucher	nrITS	Needly	rbcL	trnK-matK	petB-petD	psbB	trnT-trnD	trnL-trnF	trnS-trnG	trnV Intron	psbD-trnT	trnC-trnD	rps4-trnS
C. macrolepis	Adams 10652, (KR 7315), Vietnam	AY380855.1	AY988269.1	HM024270.1	HM023982.1	HM024078.1	HM024175.1	HM024449.1	AY988171.1	HM024618.1	HM023887.1	*KP177874	*KP177865	HM024355.1
C. decurrens	Adams 10297, Oregon, USA	AY380854.1	AY988268.1	HM024269.1	HM023981.1	HM024077.1	HM024174.1	HM024448.1	AY988170.1	HM024617.1	HM023886.1	*KP177873	*KP177864	HM024354.1
C. atlantica	Adams 8429, Morocco	AY988367.1	AY988280.1	HM024275.1	HM023987.1	HM024083.1	HM024180.1	HM008344.1	AY988182.1	JQ740558.1	HM023892.1	JQ740535.1	JQ740511.1	HM024360.1
C. dupreziana	Adams 8432, (ex Hillier Gardens), Alger	AY988375.1	AY988290.1	AY988243.1	AY988342.1	NA	NA	NA	AY988191.1	JQ740559.1	NA	JQ740536.1	JQ740512.1	NA
C. funebris	Adams 8139, (KR ex Hubei), China	AY988377.1	AY988292.1	AY988245.1	HM023991.1	HM024087.1	HM024184.1	HM008329.1	AY988194.1	HM008346.1	HM023896.1	*KP177879	*KP177871	HM024364.1
C. jiangeensis	Adams 9300, (ex Wang 026A), Tibet	AY988382.1	AY988298.1	AY988249.1	HM023993.1	HM024089.1	HM024186.1	NA	AY988199.1	HM008348.1	HM023898.1	*KP177880	*KP177872	HM024366.1
C. cashmeriana	Adams 8125, (ex Hillier), England	AY988372.1	AY988286.1	AY988240.1	HM023988.1	HM024084.1	HM024181.1	NA	AY988187.1	NA	HM023893.1	*KP177881	*KP177866	HM024361.1
C. nootkatensis	Adams 9086, Washington, USA	KJ849660.1	AY988304.1	HM024268.1	HM023980.1	HM024076.1	HM024173.1	HM024531.1	AY988207.1	JQ740538.1	HM023885.1	JQ740514.1	JQ740490	HM024353.1
X. vietnamensi	Adams 10142, Vietnam	AY380877.1	AY988329.1	HM024352.1	HM024074.1	HM024170.1	HM024267.1	HM008343.1	AY988229.1	JQ740539.1	HM023979.1	JQ740515.1	JQ740491	HM024447.1
H. bakeri	Adams 9362, California, USA	AY988369.1	AY988283.1	AY988237.1	HM023999.1	HM024095.1	HM024192.1	HM008340.1	AY988184.1	JQ740540.1	HM023904.1	JQ740516.1	JQ740492	HM024372.1
H. arizonica	Adams 9378, Arizona, USA	U77962.1	AY988278.1	AY988253.1	HM023998.1	HM024094.1	HM024191.1	HM024456.1	AY988181.1	JQ740552.1	HM023903.1	JQ740529.1	JQ740505	HM024371.1
H. macrocarpa	Adams 11460, California, USA	KJ849658.1	AY988301.1	HM024284.1	HM024005.1	HM024101.1	HM024198.1	HM024462.1	AY988204.1	HM024631.1	HM023910.1	JQ740520.1	JQ740496	HM024378.1
J. communis	Adams 7846, Sweden	AY988396.1	AY988314.1	HM024297.1	HM024019.1	HM024115.1	HM024212.1	HM024476.1	GQ301207.1	HM024645.1	HM023924.1	*KP177875	*KP177867	HM024392.1
J. excelsa	Adams 8785, Greece	HM001195.1	NA	HM024303.1	HM024025.1	HM024121.1	HM024218.1	HM024482.1	HM024565.1	HM024651.1	HM023930.1	*KP177877	*KP177869	HM024398.1
J. virginiana	Terry 139, Texas, USA	EU277699.1	NA	HM024343.1	HM024065.1	HM024161.1	HM024258.1	HM024522.1	HM024605.1	HM024691.1	HM023970.1	*KP177878	*KP177870	HM024438.1
J. osteosperma	Terry 058, Utah, USA	AY988400.1	AY988320.1	HM024318.1	HM024040.1	HM024136.1	HM024233.1	HM024497.1	HM024580.1	GU223325.1	HM023945.1	JQ740519.1	JQ740495.1	HM024413.1
J. drupacea	Adams 8795, Greece	AY380872.1	AY988317.1	HM024301.1	HM024023.1	HM024119.1	HM024216.1	HM024480.1	JF950995.1	HM024649.1	HM023928.1	*KP177876	*KP177868	HM024396.1

Table 1. Voucher and GenBank accession data for taxa used in this study. Accession numbers with an asterisk (*) are for sequences published in this study.

Hypothesis	logL	Difference	Kishino-Hasegawa	Shimodaira-Hasegawa	Likelihood Weight
C(J,HCX)	-18669.9	18.2	0.05 (-)	0.06 (+)	0.03 (-)
J(C,HCX)	-18653.1	1.4	0.30 (+)	0.61 (+)	0.32 (+)
HCX(J,C)	-18651.7	Best	1.00 (+)	1.00 (+)	0.65 (+)

Table 2. Results from maximum likelihood analysis testing the fit of the three possible J-C-HCX topologies to the chloroplast data. p-values are given under the test name and + indicates inclusion in the confidence set.

First comprehensive report on the composition of the leaf volatile terpenoids of *Pinus contorta* vars. *contorta, latifolia* and *murrayana*

Robert P. Adams
Biology Department, Baylor University, Waco, TX 76798 robert_adams@baylor.edu

Thomas A. Fairhall
953 U Street, Port Townsend WA 98368

Gay Hunter
Museum Curator, Olympic National Park, 600 East Park Ave., Port Angeles, WA 98362

and

Chauncey Parker
11643 Norse Ave., Truckee, CA 96161

ABSTRACT

The first comprehensive report on the compositions of the volatile leaf terpenoids of *Pinus contorta* var. *contorta,* var. *latifolia and* var. *murrayana* are presented. The volatile leaf oils of vars. *contorta* and *latifolia* were nearly identical, dominated by β-phellandrene (54.6, 45.1%), with moderate amounts of β-pinene (8.7, 10.3%), α-phellandrene (4.9, 1.7%), α-pinene (3.7, 3.4%), myrcene (1.9, 2.1%), δ-3-carene (0.9, 11.5%), α-terpinene (3.7, 1.6%) and terpinolene (3.7, 2.0%). With the exception of δ-3-carene (0.9, 11.5%), they differ only in minor compounds: camphor (0.2, trace), methyl chavicol (none, 0.4%), (E)-anethole (none, 0.5%),dodecanoic acid (0.6, trace), and sandaracopimarinal (0.3, trace). The oil of var. *murrayana* is considerably different from that of vars. *contorta* and *latifolia*. The volatile leaf oil of var. *murrayana* is dominated by β-pinene (38.1%) and β-phellandrene (18.2%) with moderate amounts of α-pinene (5.2%), myrcene (1.8%) and δ-3-carene (5.9%) and 7 unique components: citronellal, citronellol, geraniol, methyl eugenol, a C_{10} -diene acetate, elemicin, and sandaracopimarinol. The major components (as percent total oil) were very variable for all three taxa. Published on-line **www.phytologia.org** *Phytologia 97(1): 76-81 (Jan 2, 2015).* ISSN 030319430.

KEY WORDS: *Pinus contorta*, var. *contorta,* var. *latifolia,* var. *murrayana,* volatile leaf oil, terpenes, composition.

Smith (1967) made an early study on the wood resin monoterpenes of lodgepole pine (*Pinus contorta*) and reported the major components were β-phellandrene (53.1-78.8%) and δ-3-carene (5.6-28.6%). Zavarin, Critchfield and Snajberk (1969) examined the inheritance of turpentine composition in *P. contorta* x *P. banksiana* hybrids. Anderson, Riffer and Wong (1969) reported the major compound in *P. contorta* wood oil was β-phellandrene (71%).

However, the first report on the volatile leaf oils of *P. contorta* (var. *latifolia*) was by Pauly and von Rudloff (1971). They found the volatile leaf oil from a population near Banff, Alberta, was dominated by β-phellandrene, 37.3% (31.0-47.5%) and β-pinene, 24.2% (8.4-43%), with moderate amounts of α-pinene, 5.4% (2.8-8.9%), myrcene, 3.5% (2.0-10.5%), δ-3-carene, 4.8% (0.1-21.5%) and α-terpineol, 4.4% (2.1-4.4%). They noted that the variation among *P. contorta* individuals was very large (compared to other conifer oils they had examined). Von Rudloff, Lapp and McMinn (1985) studied variation in the leaf oils from young and old lodgepole pine from different moisture regimes, Prince George, BC. In addition to the typical terpene patterns previously seen in lodgepole, they found some

old trees without resin canals, that had a new terpene profile and very low oil yield (0.1%). No terpene differences were found in young trees from upland or bog sites or from dry and wet sites.

Von Rudloff and Lapp (1987) surveyed the leaf oils of P. *contorta* vars. *contorta* and *latifolia*, *murrayana* and *bolanderi* from 111 different population sites in British Columbia, Alberta and the northwestern United States. They found hybridization between vars. *contorta* and *latifolia* in western BC and on Vancouver Island, BC. The oils from populations of putative *P. c.* var. *murrayana* from northern California and Oregon were found to be similar to that of var. *contorta*; although in this area, the two varieties seem to intergrade.

At the same time, Croteau, et al. (1987) began research on the biochemistry of oleoresins with an emphasis on the bark beetle induction of the synthesis of defense chemicals (terpenes). This has led to numerous papers on the effects of bark beetles on resin production (see Ott et al., 2011; Wallis, et al. 2011). Recently, the search for the gene sequence of terpene synthase (TPS) genes has exploded by the work of Bohlmann's lab (see review by Keeling and Bohlmann, 2006, and Foster et al. 2013).

Because the early work by von Rudloff's lab did not report comprehensive oil analyses, it seems an appropriate time to re-analyze the leaf volatile oils of *Pinus contorta* var. *contorta,* var. *latifolia and* var. *murrayana* using modern GC/MS/computer technology (Adams, 2007).

MATERIALS AND METHODS

Leaf samples were collected from *Pinus contorta* var. *contorta*: Fort Worden State Park, Port Townsend, WA, on sand. 48° 08' 27" N; 122° 45' 38" W. elev. 25 ft, 20 Nov 2104, Jefferson Co., WA, Coll. *Tom Fairhall 1-5,* Lab Acc. *Robert P. Adams, 14477-14481.*
Pinus contorta var. *latifolia*: Deer Ridge, Olympic Mountains, 47° 56' 45.5" N; 123° 12' 58.4" W. elev. 4291 ft, 20 Nov 2104, Jefferson Co., WA, Coll. *Tom Fairhall & Gay Hunter 1,* Lab Acc. *Robert P. Adams 14482*; Deer Trail Ridge, Olympic Mountains, with *J. jackii*, 47° 56' 43.2" N; 123° 13' 53.5" W. elev. 4885 ft, 20 Nov 2104, Jefferson, Co., WA, Coll. *Tom Fairhall & Gay Hunter 2-5*, Lab Acc. *Robert P. Adams 14483-14486.*
Pinus contorta var. *murrayana*: abundant, south side of Donner Pass Road (Lincoln Hwy), 0.25 air mi. east of Donner Pass, 39° 19' 03.24" N; 120° 19' 17.64" W. elev. 6863 ft, 28 Sep 2014, Placer Co., CA, Coll. *Chauncey Parker BA 1-10*, Lab Acc. *Robert P. Adams 14450-14459.*
Voucher specimens are deposited in the herbarium, Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The composition of the leaf oils are given in table 1. The volatile leaf oils of vars. *contorta* and *latifolia* were nearly identical, dominated by β-phellandrene (54.6, 45.1%), with moderate amounts of β-pinene (8.7, 10.3%), α-phellandrene (4.9, 1.7%), α-pinene (3.7, 3.4%), myrcene (1.9, 2.1%), δ-3-carene (0.9, 11.5%), α-terpinene (3.7, 1.6%) and terpinolene (3.7, 2.0%). With the exception of δ-3-carene (0.9, 11.5%), they differ only in minor compounds: camphor (0.2, trace), methyl chavicol (none, 0.4%), (E)-anethole (none, 0.5%), dodecanoic acid (0.6, trace), and sandaracopimarinal (0.3, trace). The oil of var. *murrayana* is considerably different from that of vars. *contorta* and *latifolia*. The volatile leaf oil of var. *murrayana* is dominated by β-pinene (38.1%) and β-phellandrene (18.2%) with moderate amounts of α-pinene(5.2%), myrcene(1.8%) and δ-3-carene(5.9%) and 7 unique components: citronellal, citronellol, geraniol, methyl eugenol, a C_{10} -diene acetate, elemicin, and sandaracopimarinol.

The major components (as percent total oil) were very variable for all three taxa. (Table 1). For vars. *contorta*, *latifolia,* and *murrayana* the ranges were: β-pinene (1.4-21.3%), (0.7-26.5%) and (27.1-46.2%), δ-3-carene (0.4-1.2%), 6.1-19.4%) and (2.8-11.0%), and β-phellandrene (39.2-61.5%), 32.5%-58.5%) and (15.9-21.9%). This agrees with the observations of Pauly and von Rudloff (1971) on *P. contorta* leaf oils.

In contrast to von Rudloff and Lapp (1987) who found the oils from populations of putative *P. c.* var. *murrayana* from northern California and Oregon to be similar to that of var. *contorta*, we found our oil from *P. c.* var. *murrayana* to be quite different from *P. c.* vars. *contorta* and *latifolia*. This may be because our samples came from Donner Pass, CA (west of Reno, NV). A site that is far to the south of the von Rudloff and Lapp (1987) samples in northern California and Oregon. Their var. *murrayana* samples may be the product of hybridization with vars. *contorta* and *latifolia*. Our var. *murrayana* population is quite disjunct from vars. *contorta* and *latifolia* with no opportunity for hybridization and appear to be typical of the variety.

Von Rudloff, Lapp and McMinn (1985) reported finding some old *P. contorta* trees with very low oil yields (0.1%). Their leaves did not contain resin canals. In this study, we found one *P. contorta* var. *contorta* tree (*14479*) with an oil yield of 0.1% and it does not appear to have resin canals. We also found one *P. c.* var. *latifolia* (*14485*) with an unusually high yield (3.45%). The 2 hr. steam distillation is estimated to remove about 30-35% of the oil. Thus, this tree is storing about 10% (dry wt) of oil in its leaves. This is about 5 times the amount of any other tree sampled and 30 times the oil in tree *14479*. .It appears *Pinus contorta* appears to be a good species for the study of TPS genes controlling the synthesis of essential oil production.

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Table 2. Comparison of leaf oil compositions of *Pinus contorta* var. *contorta,* var. *latifolia and* var. *murrayana.* Compounds in bold face appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. KI is the Kovat's Index using a linear calculation on DB-5 column.

KI	compound	*contorta*	*latifolia*	murrayana
846	(E)-hexenal	0.4	0.1	t
850	(3Z)-hexenol	1.3	0.3	0.2
921	tricyclene	0.1	0.1	t
924	α-thujene	0.2	0.2	0.1
932	α-pinene	3.7(2.4-4.3)	3.4(1.9-5.3)	5.2(2.8-6.6)
946	camphene	1.2(0.7-2.7)	0.7(0.4-1.3)	1.2(0.7-2.2)
969	sabinene	0.9	0.7	0.1
974	**β-pinene**	**8.7(1.4-21.3**	**10.3(0.7-26.5)**	**38.1(27.1-46.2)**
988	myrcene	1.9(1.5-2.1)	2.1(1.8-2.40	1.8(1.3-2.4)
1002	**α-phellandrene**	**4.9(1.4-4.0)**	**1.7(1.5-4.6)**	**0.7(0.2-0.9)**
1008	**δ-3-carene**	**0.9(0.4-1.2)**	**11.5(6.1-19.4)**	**5.9(2.8-11.0)**
1014	α-terpinene	3.7(2.1-8.9)	1.6(1.1-6.9)	0.7(0.5-2.7)
1020	p-cymene	t	t	0.1
1025	**β-phellandrene**	**54.6(39.2-61.50**	**45.1(32.6-58.5)**	**18.2(15.9-21.9)**
1032	(Z)-β-ocimene	0.8	2.4	2.7
1044	(E)-β-ocimene	t	0.2	0.1
1054	γ-terpinene	0.8(0.6-1.7)	0.5(0.5-2.7)	0.3(0.3-1.4)
1065	cis-sabinene hydrate	t	t	t
1086	terpinolene	3.7(3.4-5.5)	2.0(1.1-3.8)	1.6(0.6-3.8)
1095	linalool	0.1	0.2	0.4
1100	n-nonanal	t	t	t
1118	cis-p-menth-2-en-1-ol	0.1	0.1	0.1
1122	α-campholenal	t	t	t
1135	nopinone	t	t	t
1136	trans-p-menth-2-en-1-ol	-	0.1	0.1
1141	camphor	0.2	t	t
1145	camphene hydrate	t	t	t
1148	**citronellal**	**-**	**-**	**0.1**
1158	trans-pinocamphone	t	t	-
1160	pinocarvone	t	0.1	-
1165	borneol	t	0.1	0.3
1174	terpinen-4-ol	0.3	0.2	0.2
1179	p-cymen-8-ol	0.3	0.1	t
1186	α-terpineol	0.2	0.3	1.6
1195	**methyl chavicol**	**-**	**0.4**	**0.3**
1195	myrtanal	t	-	-
1195	myrtanol	t	-	-
1207	trans-piperitol	t	t	-
1223	citronellol	-	-	t
1232	thymol, methyl ether	-	t	-
1238	cumin aldehyde	t	-	-
1249	geraniol	-	-	t
1249	piperitone	t	t	-
1282	**(E)-anethole**	**-**	**0.5**	**0.2**
1284	**bornyl acetate**	**0.6(0.1-2.0)**	**0.2(0.1-0.4)**	**t**
1289	thymol	t	-	-
1293	2-undecanone	t	0.1	0.2
1315	(2E,4E)-decadienal	t	-	-
1335	δ-elemene	t	t	-
1345	α-cubebene	t	t	-
1350	citronellyl acetate	t	t	-
1374	α-copaene	-	t	t
1379	**geranyl acetate**	**t**	**t**	**0.5**
1389	β-elemene	t	t	t
1396	duvalene acetate	t	-	-
1400	cis-sibirene	t	t	-
1403	methyl eugenol	-	-	t
1417	(E)-caryophyllene	t	t	0.2
1439	aromadendrene	t	t	-

KI	compound	*contorta*	*latifolia*	murrayana
1442	**6,9-guaiadiene**	**1.7(0.4-4.0)**	**0.6(0.2-0.9)**	**-**
1449	trans-sibirene	t	t	-
1454	(E)-β-farnesene	-	t	-
1467	**C10-diene, acetate, 43,54,67,196**	**-**	**-**	**0.5(0.3-1.5)**
1475	trans-cadina-1(6),4-diene	-	t	-
1478	γ-muurolene	t	0.2	0.2
1480	germacrene D	0.2	0.4	0.6
1489	β-selinene	0.1	0.1	t
1493	epi-cubebol	-	0.3	-
1500	bicyclogermacrene	0.8	0.6	0.8
1500	α-muurolene	t	0.4	0.3
1513	γ-cadinene	0.5	1.4	1.0
1522	δ-cadinene	0.8(0.4-2.0)	2.3(2.2-4.4)	2.1(0.4-3.4)
1537	α-cadinene	t	0.1	0.1
1555	elemicin	-	-	0.2
1561	(E)-nerolidol	-	-	0.1
1565	**dodecanoic acid**	**0.6**	**t**	**-**
1574	**germacrene-D-4-ol**	**0.3**	**1.5**	**2.4(0.5-4.0)**
1577	**spathulenol**	**-**	**-**	**0.2**
1590	globulol	t	t	-
1627	1-epi-cubenol	-	0.1	-
1638	epi-α-cadinol	0.1	0.4	0.5
1640	epi-α-muurolol	0.1	0.4	0.5
1644	α-muurolol	t	0.1	0.2
1652	**α-cadinol**	**0.1**	**0.8**	**1.4**
1671	tetradecanol	t	-	-
1722	(2Z,6E)-farnesol	0.2	0.2	0.4
1758	tetradecanoic acid	0.1	-	-
1814	hexadecanol	t	-	-
1944	pimara-8(14),15-diene	t	-	0.2
1987	iso-pimara-7,15-diene	t	t	0.2
2056	manool	0.1	0.5(0.1-1.8)	0.6(0.1, 1.6)
2087	abietadiene	t	t	-
2149	abienol	t	t	-
2184	sandaracopimarinal	0.3	t	1.0
2221	diterpene, 51,187,257,286	0.4	0.2	0.8
2237	diterpene, *43*,91,*133*,286	0.3	0.4	0.6
2269	**sandaracopimarinol**	**-**	**-**	**0.2**
2274	dehydro abietal	t	t	-
2313	abietal	t	t	-

A review and update of the genus *Sapromyces* (Straminipila: Oomycota)

Will H. Blackwell, Peter M. Letcher and Martha J. Powell
Dept. Biological Sciences, Univ. Alabama, Tuscaloosa, AL 35487

ABSTRACT

Sapromyces—a freshwater saprotrophic genus of Oomycota, traditionally classified in the Leptomitales, more recently in the Rhipidiales—has received scant taxonomic attention since Sparrow's (1960) revised *Aquatic Phycomycetes*. Sparrow recognized the two species of *Sapromyces* well-established at that point, *S. elongatus* and *S. androgynus*; a third species, *S. indicus*, was included, but not incorporated in his key, apparently because information did not reach Sparrow in time to completely readjust text. Dick (2001) recognized these three species—providing nomenclatural information, but not distinguishing features. A goal of our investigation was to assess *S. indicus* Iyengar et al., and, accepting this species, include it in a revised key. We conclude that *Sapromyces* should indeed contain three species, *S. elongatus*, *S. androgynus* and *S. indicus*; a fourth possible species, *S. dubius* Fritsch, based on sparse, uncertain material (initially described, but not named, by Reinsch), is considered "doubtful." *Sapromyces reinschii* (J. Schröt.) Fritsch is treated as a synonym of *S. elongatus*. Authorship of the binomial combination *Sapromyces elongatus* was incorrectly attributed; it should be *S. elongatus* (Cornu) Thaxt. Thaxter is sole author of *S. androgynus*. The original source (which of two publications?) of the basionym of *S. elongatus* (*Rhipidium elongatum*) required clarification. The generic name *Sapromyces* invited attention because of two preceding synonyms, and a possible later homonym. We report our find of *Sapromyces* in Alabama—a probable identity to species—and discuss aspects of morphology: Form and mode of growth, potential vegetative reproductive structures, and absence of antheridia. Published on-line **www.phytologia.org** *Phytologia 97(2): 82-93 (April 1, 2015).* ISSN 030319430.

KEY WORDS: Antheridium, *Apodachlya*, Oogonium, Oomycetes, *Rhipidium*, Species, Sporangium.

Although genera of particular groups of Oomycota (e.g., "water-molds" of the Saprolegniales, and plant pathogens of the Peronosporales) are well known and frequently investigated organisms, many other genera (especially in lesser known orders) have remained obscure. In recent times, though, some of these more poorly understood representatives have received at least a modicum of taxonomic study (e.g., Blackwell 2010, 2011; Blackwell et al., 2013, 2014; Pereira and Vélez, 2004). *Sapromyces*, considered here, is an incompletely known genus, receiving perhaps even less attention than certain other poorly known Oomycetes since it is fundamentally saprotrophic (i.e., not "problematic" as a parasite). *Sapromyces* has been considered a member of the Leptomitales (Kanouse, 1927; Sparrow, 1960), an order distinguished from the Saprolegniales (which they may generally resemble) by hyphae that are often distinctly constricted at intervals. Although fundamentally coenocytic (characteristic of Oomycetes generally)—and not as prone to form sporadic cross-walls, as encountered in members of the Saprolegniales (e.g., in association with sporangia)—members of the Leptomitales may develop restrictive plugs of "cellulin" granules, internally, associated with hyphal constrictions (cf. Sparrow, 1960; Alexopolous, 1962; Dick, 1973), and thus form what may be termed "pseudo-cells" (segments that resemble and perhaps function as cell units). There is a trend in the Leptomitales to form oogonia which contain but a single oosphere. Associatedly, oogonial contents of members of the Leptomitales are often differentiated not only into ooplasm (which develops into the oosphere, or "egg," as in Saprolgeniaceae) but also (in distinction to Saprolegeniaceae) into a surrounding periplasm, leaving little "free-space" between the oogonial wall and developing oosphere (cf. Alexopoulos, 1962, p. 150). In the Leptomitales generally (in connection with possession of periplasm) the oospore matures centripetally—in contrast to a typically centrifugal development of oospores of Saprolegniaceae (Dick, 1969).

Within the Leptomitales (cf. Sparrow, 1960), forms such as *Leptomitus* and *Apodachlya* were classified in the Leptomitaceae (thallus relatively undifferentiated), whereas *Sapromyces*, *Rhipidium*, *Araiospora*, and *Mindeniella*, were placed in the Rhipidiaceae (thallus comparatively differentiated). *Sapromyces* is distinguished, though, from other genera of Rhipidiaceae by a basal (attachment) pseudo-cell not strikingly morphologically differentiated from other thallus "cells." Dick (2001) included genera of Rhipidiaceae (including *Sapromyces*, which can bear a superficial resemblance to *Leptomitus*) in an expanded concept of the grouping, i.e., the Rhipidiales, in his subclass Rhipidiomycetidae; *Leptomitus* and *Apodachlya*, retained in the Leptomitales, were transferred to subclass Saprolegniomycetidae. Dick's (2001) separation, of these seemingly similar groups of pseudo-fungi into different subclasses, requires scrutiny, but is not greatly dissimilar to Sparrow's (1976) placement of the Rhipidiaceae in the "Peronosporacean galaxy" and the Leptomitaceae in the "Saprolegnian galaxy." Whereas certain molecular-genetic information (Sekimoto et al., 2007; Beakes and Sekimoto, 2009; Beakes et al., 2012) appears to support such a broad separation (e.g., of *Apodachlya* from *Sapromyces*), other data (Riethmüller et al., 1999; Lara and Belbahri, 2011) may suggest closer relationship. Beakes et al. (2014, p. 51 vs. p. 53) bring to light what seem to be, at least in part, conflicting molecular outcomes. Further resolution of molecular data, including additional sampling diversity of Oomycetes (as, for example, suggested by Beakes et. al, 2014), is necessary to be confident of relationships between members of the Leptomitales and those of the Rhipidiales (and, for that matter, of genera within each order).

HISTORICAL TAXONOMIC INFORMATION ON *SAPROMYCES*

Sapromyces Fritsch (1893) is the correct name for this genus of rhipidialean Oomycetes, because two prior names—*Naegelia* Reinsch (1878), and *Naegeliella* J. Schröt. (date usually given as1893, although the separate of *Die Natürlichen Pflanzenfamilien* containing *Naegeliella* was probably published in 1892)—were both preoccupied (already employed for genera unrelated to *Sapromyces*, or to each other). Consequently, these similar names (*Naegelia* and *Naegeliella*), in application to *Sapromyces*, can only be interpreted as illegitimate synonyms. The genus *Sapromyces* is at present considered to contain three species (see Key, and Figs.1-8), as is subsequently discussed.

In seeming nomenclatural complication, *Sapromyces* Sabin (1941, see Lit. Cited) was erected as the generic name for certain strains of bacteria of the Pleuropneumonia group found in sewage. Sabin's (1941) name was accepted (Edward and Freundt, 1969), but soon rejected (Edward and Freundt, 1970) on grounds of prior existence of the Leptomitalian genus by the same name (*Sapromyces* Fritsch, 1893). It might seem that Edward and Fruendt were correct in 1969, to the extent that a name published under one code of nomenclature (e.g., the "bacteriological" or "prokaryotic" code) is generally allowed to stand, regardless of an identical name recognized under another code (e.g., the "botanical code," or its current name); however, their stance in 1970 could also be supported in that the bacteriological code may not consider such a bacterial name (i.e., a "later homonym" of a "fungal name") legitimate. Regardless, Sabin's (1941) name has no effect on Fritsch's (1893) name.

Two species of *Sapromyces* were recognized by Coker and Matthews (1937), *Sapromyces androgynus* Thaxt. (1896) and *S. elongatus* (Cornu) Coker in Coker & V. D. Matthews (1937; see Lit. Cited). The source of the epithet "*elongatus*" (*Rhipidium elongatum* Cornu) was recognized by Coker and Mathews as Cornu (1872). These species, authorships, and publication dates were subsequently recognized by Sparrow (1960) and Dick (2001). In seeming contradiction, though, whereas Dick (2001) credited the combination *Sapromyces elongatus* to Coker in information provided on this species, he also credited the same combination (in the same publication)—when indicating typfication of genus *Sapromyces*—to Thaxter (1896). As noted currently in *Index Fugorum* (online update), it was indeed Thaxter who first made the combination of Cornu's "*elongatum*" with *Sapromyces*; the citation should thus properly be *Sapromyces elongatus* (Cornu) Thaxt., not (Cornu) Coker. Additionally, *Index Fungorum* lists the original publication of the epithet "*elongatum*" (i.e., *Rhipidium elongatum*) as Cornu,

1871 (a different publication than Cornu, 1872). Thaxter (1896) focused on Cornu's 1872 publication, but also cited Cornu (1871) in noting four species of *Rhipidium*—including *R. elongatum*, which Thaxter believed (based on information in Cornu) did not belong in *Rhipidium*; Thaxter considered that *R. elongatum* was more appropriately placed in genus *Sapromyces*. Adequate descriptive information of species (of "*Rhipidium*") is provided in Cornu (1871), very similar to that presented by him in 1872. There is thus no reason not to regard Cornu's 1871 publication as the source of valid publication of *Rhipidium elongatum* (basionym of *Sapromyces elongatus*), and we so accept it herein.

Although *Sapromyces* (Fritsch, 1893) stands as the correct name of the genus, Fritsch did not employ the earliest available epithet ("*elongatum*" of Cornu) for the original species—using, rather, the binomial *Sapromyces reinschii* (J. Schröt.) Fritsch (based on *Naegeliella reinschii* J. Schröt. 1892/1893). Since "*reinschii*" is not the earliest epithet, *S. reinschii* has, by most authors, been regarded as a synonym of *Sapromyces elongatus*. As indicated, the correct combination—*Sapromyces elongatus* (Cornu) Thaxt.—was provided by Thaxter (1896, p. 326). It was in this publication that Thaxter (p. 329) described *Sapromyces androgynus* as a new species. In addition to recognizing *S. androgynus* and *S. elongatus*, Thaxter, perhaps surprisingly, also recognized *S. reinschii* (p. 326). Thaxter indicated that *S. androgynus* may be distinguished from *S. reinschii* by smaller stature, but provided no insight into a distinction between *S. reinschii* and *S. elongatus*. Equally perplexing, Dick (2001), though listing *S. reinschii* as synonym of *S. elongatus*, also listed *S. reinschii* under "excluded species." A problem is that *S. reinschii* was based (Reinsch, 1878; Schröter, 1892/1893) only on asexual material (see Reinsch, 1878, re: species "1" of "*Naegelia*," figs. 1-6 of his plate 15; see also our Figs. 9-10)—these specimens being, however, at least not inconsistent with *S. elongatus* (although sporangia of *S. reinschii*, as originally illustrated, exhibit a more consistently "whorled" pattern than is perhaps usually the case in specimens of *S. elongatus*). After reviewing original literature (Cornu, 1871, 1872; Reinsch, 1878), and although its disposition remains somewhat uncertain, we do not find sufficient evidence to consider *S. reinschii* a distinct species. Sparrow (1932) at first recognized *S. reinschii*, but later (1943) employed the earliest epithet, as correct for this species, i.e., *S. elongatus*. We therefore (albeit perhaps by default) regard *S. reinschii* as a synonym of *S. elongatus* (as in Coker and Matthews, 1937; Sparrow, 1960; Dick, 2001).

In a detailed account of *Sapromyces*, Sparrow (1960) provided a key to the two species of the genus definitely established at that time, *S. elongatus* and *S. androgynus*. Sparrow seemingly accepted a third species (*Sapromyces indicus* M. O. P. Iyengar et al., 1955), but did not include it in his key, since it apparently did not come to light in time for a full account in the second edition of *Aquatic Phycomycetes*. Dick (2001) recognized *S. indicus*, but did not indicate its distinction from other species. A fourth possible species of *Sapromyces*, *S. dubius* Fritsch (1893), based on an unnamed species of Reinsch (1878)—designated by Reinsch as species "2" (of "*Naegelia*")—was considered a synonym of *S. elongatus* by Coker and Matthews (1937). However, Sparrow (1943), though initially tentatively considering *S. dubius* a synonym of *S. elongatus*, later excluded *S. dubius* from *Sapromyces*, coming to believe that it represented a fragment of a *Rhipidium* species (Sparrow, 1960). Dick (2001) likewise excluded *S. dubius* from *Sapromyces*, but without comment on its identity. We concur to the extent of considering *S. dubius* questionable, and do not include it in our key. However, given the paucity of the original material, and Reinsch's sparse illustration (1878, figs. 7-11 of his plate 15; see also our Fig. 11), we are uncertain of its determination, and unable, with assurance, to either include it in or exclude it from *Sapromyces*. Perhaps future collections will resolve the disposition of "*Sapromyces dubius*."

The situation now, virtually unchanged since Sparrow (1960), is that three species of *Sapromyces*—*S. elongatus*, *S. androgynus* and *S. indicus*—are recognized. Little information has accrued directly useful to solution of taxonomic problems in the genus. Because Sparrow, of necessity, hastily included *S. indicus* M. O. P. Iyengar et al. (1955) in the genus, what falls to our study (other than matters of nomenclature) is to evaluate *S. indicus*, and, if accepting its distinctiveness, include it in a revised key to species. Additionally, we present our find of *Sapromyces*—a new record in Alabama, adding

substantially to the geographic record in the USA (cf. Sparrow, 1960). Although not all structures of the life cycle were observed in these specimens, we indicate a probable determination to species. Particular aspects of morphology are discussed, that may contribute to a better understanding of *Sapromyces*.

TAXONOMIC SUMMARY

Sapromyces Fritsch, 1893 (Oomycota)
Naegelia Reinsch, 1878; non L. Rabenhorst, 1844 (Fungi incerti sedis)
Naegeliella J. Schröt., 1893; non C. Correns, 1892 (Orchidaceae)

Type species: *Sapromyces elongatus* (Cornu) Thaxt. (1896); based on *Rhipidium elongatum* Cornu (1871). Synonym: *Sapromyces reinschii* (J. Schröt.) Fritsch (1893); based on *Naegeliella reinschii* J. Schröt. (1892/1893); in turn based on an unnamed species of *Naegelia* described by Reinsch (1878, see pp. 289-291, 298, and figs. 1-6 of his plate 15), designated by Reinsch as species "1."

In addition to the relatively undifferentiated basal pseudo-cell, *Sapromyces* may be distinguished from genera of traditional Rhipidiaceae (and related Leptomitalean genera) by a combination of features, none perhaps singularly distinctive; these features include: An elongate growth form (obtained by predominantly terminal budding), and a sometimes umbel-like pattern of hyphal branching; hyphal segments that are often elongate, clavate, or sometimes more generally broadened; sporangia that are frequently pedicillate (a consequence of formation by constriction and budding), the sporangia often becoming elongate and cylindrical or more broadly ovate in form, and sometimes clustered or in apparent whorls on or near a hyphal tip; membrane surrounding the emerging zoospores typically rupturing rapidly to potentially free the zoospores; spiral rotation of an antheridial branch; attachment of the antheridium at or near the apex of the oogonium; and obpyriform to spherical oogonia, subject to external encrustation.

Key to Species of *Sapromyces* (with notes on distribution, collection and morphology)

1. Oospore wall merely roughened, uneven, or sometimes undulate or with low protuberances; mature sporangia often exceeding 80 µm in length; basal pseudo-cell from 115 to as much as 1200 µm long and which may constitute a significant proportion of the thallus, relatively thin-walled, often branching in a hapteroid fashion at the base; species androgynous or diclinous.

    2. Antheridial branches short, androgynous (borne on oogonial branches); oogonium obpyriform; basal "cell" 115-250 µm long……*S. androgynus* Thaxt. (1896)

    2. Antheridial branches long, winding, diclinous (occurring on non-oogonial branches); oogonium spheroidal; basal "cell" 300-1200 µm long……..*S. elongatus* (Cornu) Thaxt. (1896)

1. Oospore wall distinctly reticulate; sporangia often less than 80 µm long; basal pseudo-cell 40-80 µm long, constituting a relatively small portion of thallus, thick-walled, pear- or vase-shaped, unbranched; species is diclinous……...*S. indicus* M. O. P. Iyengar et al. (1955)

For useful descriptive accounts of the three recognized species of *Sapromyces*, see Coker and Mathews (1937) and Sparrow (1960) for *S. elongatus* and *S. androgynus*, and Iyengar et al. (1955) for *S. indicus*; see also our illustrations (Figs. 1-8). An extensive nomenclatural listing and typfication of these species is available in Dick (2001). Evaluation of literature (and illustrations) of *S. indicus* Ineygar et al. (1955) indicates that it is a distinct taxon, deserving recognition equal to *S. elongatus* and *S. androgynus*.

Distributional note: *Sapromyces indicus* was reported from fallen leaves in stream-water in the Kambakkam Hills, 60 miles north of Madras (Iyengar et al., 1955). Both *Sapromyces elongatus* and *S.*

androgynus are geographically widespread (Sparrow, 1960), being known, for example, from various locations in Europe and North America. Yet, confirmed reports are infrequent, particularly for *S. androgynus* (Sparrow, 1960, p. 886); this is not to suggest, though, that these forms may not be locally common (Thaxter, 1896, re: *S. androgynus*). The relative rarity of reports of *Sapromyces* is probably more a reflection of lack of collection than infrequency of occurrence. Reports are usually from twigs, leaves, or small fruits floating in shallow water. "Baits" (e.g., pear or apple) do not seem to have been used in some cases; if baiting were more often utilized, reports of *Sapromyces* would likely become more common. Czeczuga et al. (2007) considered both *S. androgynus* and *S. elongatus* rare, but by using baits of various fruits and seeds (in bodies of water in eastern Poland) they were able to isolate both species.

Our collection (Figs. 13-25). Our specimens of *Sapromyces* were obtained by baiting on small slices of pear in a water collection (containing floating twigs and privet fruit) from a stagnant feeder creek to Northwoods Lake; Northport, Tuscaloosa Co., Alabama (collection WB#302). This collection adds significantly to an already geographically broad (if not necessarily common) distribution of the genus *Sapromyces* in the United States, Canada, Central America, Europe, Asia and Australia (cf. Sparrow, 1960). Most USA collections are northern. Records from the southeastern USA are sparse; Sparrow (1932) reported a probable (but sterile) collection of *S. reinschii* (= *S. elongatus*) from North Carolina by J. N. Couch; we find no previous report of *Sapromyces* from Alabama. Because of a predominantly asexual state, it was not possible to definitively determine our specimens to species; however, their morphology—combination of the ovate form of sporangia (cf. Emerson, 1958, his fig. VII, 3; and our Figs. 13-15, 17) and extensive, flocculent thallus, with the smooth, rounded form of apparent, young oogonia—is more consistent with *Sapromyces elongatus* than *S. androgynus* or *S. indicus*. The fact that antheridial branches were not observed (as they probably would have been in a monoecious form like *S. androgynus*) is possibly indicative not only of a diclinous condition (as in *S. elongatus*), but of an organism that may occur in distinct sexual strains (i.e., that is possibly heterothallic, cf. Sparrow, 1932, in discussing "S. reinschii")—and, thus, that is potentially dioecious (occurring as distinct male and female "plants"). Specimens in our collection, remaining in a de facto vegetative state, eventually degenerated in water culture in spite of attempts at culture transfer. Additional finds of *S. elongatus* could confirm its heterothallic nature; however, the fact that Bishop (1940) noted "latent maleness" (ability to eventually initiate antheridial formation) in certain female strains of "*Sapromyces reinschii*" (= *S. elongatus*), maintained in culture, would suggest that homothallism (and monoecism) cannot be entirely ruled out in this species, under all culture or environmental conditions. Also, Sparrow (1960, p. 860) noted that some strains of *S. elongatus* remained sterile, regardless of exposure to other, sexual strains. The fact that sporangia in our specimens maintained an ovate, "juvenile" form (e.g., our Fig. 14)—never fully elongating (cf. Emerson, 1958, his fig. VII, 2) and never observed to form zoospores—could indicate that our specimens represent not only an asexual strain, but one that is predominantly "vegetative" as well.

Unusual Aspects of Morphology (Our specimens): Some hyphal pseudo-cells observed were swollen (Figs. 16, 18, 19) and possessed large, refractive granules (cf. Dick, 1973; see our Fig. 19); these hyphal units can assume odd, uneven shapes (Figs. 18, 19), and could detach from the thallus (or else the thallus may fragment into segments or "pieces," Fig. 18). Such features suggest not only a depauperate state of the thallus (in less than optimal environmental conditions), but also potential vegetative reproduction by "gemmae" (asexual reproductive bodies). The production of possible vegetative reproductive bodies in *Sapromyces* has seemingly not been previously reported. Additionally, the fact that structures which were apparently oogonia (Fig. 22) initiated formation, but did not develop further (remaining juvenescent), suggests the possibility (discussed, in part, above) that the presence of a male (antheridial) strain of this species may be required in the micro-environment for continued oogonial development (and subsequent oosphere and oospore formation)—a supposition somewhat supported by information in Bishop, 1940 (extending work begun by Jordon, in Weston, 1938), and Sparrow (1960, pp. 859-861). *Sapromyces* is difficult to culture through its life cycle. Emerson (1958) reported a lack of success in determining conditions, in pure culture, that would successfully induce gametangial formation. Gleason and Unestam

(1968), studying terminal respiration (cytochromes) of various Leptomitales, also noted difficulties in culturing *Sapromyces* (*S. elongatus*) under certain conditions (e.g., reduced oxygen tension levels). We suspect that difficulties we encountered in maintaining water cultures (substrate added) over a period of time possibly arose from previous environmental depletion, e.g., reduced oxygen levels, in the initial, natural collection (and thus specimens, already stressed, which were unable to recover satisfactorily).

Growth Form: Growth form is of general interest in genera of the traditional Leptomitales. The terms "monopodial" and "sympodial" were often used to describe thallus patterns. Sparrow (1960) considered *Sapromyces* and *Apodachlya*, for example, to be monopodial (growing by supposed dominance of a main axis). However, Thaxter (1896) seemed to indicate that *Apodachlya* could be sympodial; certainly, *Apodachlya* is more prone to lateral branching than some other Leptomitalean forms (cf. Dick, 1973). Fitzpatrick's illustration (1930, p. 176) of *Sapromyces androgynus* also suggests possible sympodial growth (e.g., growth by overtopping by lateral braches). Iyengar et al. (1955, p. 143) indicated that thallus growth of *S. indicus* could be sympodial, which seems odd, since they also indicated (p. 140) that its "growth is always terminal." The problem with terms like monopodial and sympodial (perhaps more clearly used in connection with higher plants and kinds of algae) is that such are difficult to apply with certainty to organisms such as leptomitalean Oomycetes (e.g., *Sapromyces*), which often grow in a somewhat irregular fashion, by an almost yeast-like budding or "pinching," from a small, usually distal, "papilla-like," segment of cytoplasm, isolated just beyond a point of constriction—see Dick, 1973, p. 155, fig. 3 of his plate I; compare also our Figs. 12 and 7, and 25 and 17. It can be difficult to say if this budding is strictly terminal (precisely apically polar), or somewhat to one side or other of the "cell" apex—bearing on how "straight" the hyphal filament will ultimately be. In our specimens of *Sapromyces*, a branch or sporangium could be terminal (Figs. 14, 15), both terminal and to the side of the hyphal apex (Figs. 13, 20, 23), or arise at the sides of the apex with no clear terminal structure (Fig. 21). The pattern can be obscured (e.g., in *Sapromyces elongatus*) by multiple budding from an apex, resulting sometimes in an umbel- or whorl-like appearance of sporangia or branches (see descriptive information in Sparrow, 1960, p. 884; and Reinsch, 1878, figs. 1, 2 of his Plate 15; and our Figs. 9 and 24). It becomes more difficult to decipher such in a form like *Rhipidium*, with numerous, almost brush-like branches arising from the surface of a broadened, flattened or irregular apex of a specialized basal cell (cf. Fitzpatrick, 1930, p. 179). It is more meaningful to discuss whether budding of branches or sporangia is generally (not precisely) from the apical ("polar") region of a "cell" (as in *Sapromyces*, *Araiospora* and, more obscurely, *Rhipidium*) or whether this may also occur from a lateral position; Dick (1973, re: figs. 1, 2 of his Plate I) used the term "nonpolar" for such lateral bud-branching, as in *Apodachlya* (seen rarely in *Sapromyces*, cf. Iyengar et al., 1955, their fig. 12; our Fig. 6). Polar vs. nonpolar development of branches of members of Leptomitales should not be confused with a bipolar development of germinating zoospores of various genera (e.g., *Sapromyces*, *Rhipidium*, *Aqualinderella*; cf. Emerson and Weston, 1967). In any event, growth form (branching pattern) of the Leptomitales is a suitable subject for future study.

Perhaps worthy of further comment—without necessarily implying special phylogenetic meaning—is the supposedly distinguishing growth pattern of *Sapromyces*. This growth form, while fundamentally elongate and distinctly "hyphal" (generally resembling *Leptomitus* and *Apodachlya*, for example), nonetheless involves a basal (attachment) "cell" (as in *Rhipidium, Araiospora* and *Mindeniella*). However, this basal unit in *Sapromyces*, though present (e.g., see our Fig. 1), is not distinctly specialized (as compared, for example, with that in *Rhipidium* and *Araiospora*, cf. figs. 66-68 in Fitzpatrick, 1930). The thallus of *Sapromyces* could perhaps be viewed as somewhat intermediate (as regards the breadth of cells and presence or degree of specialization of a basal cell) between seemingly relatively undifferentiated, "filamentous" (or "myceliar") forms of the Leptomitaceae (*Leptomitus* and *Apodachlya*) and more differentiated or reduced ("monocentric") forms of the Rhipidiaceae (*Rhipidium*, *Araiospora*, *Mindeniella*, and *Aqualinderella*). Emerson and Weston (1967) suggested that a gradational series of forms appears to exist within the Leptomitales—in which *Sapromyces* might represent a connecting link (cf. Dick, 1973, p. 146) between more "typical" representatives of respective families of

the original order (see also Iyengar et al., 1955, p. 143). However, the reality of *Sapromyces* as an actual connecting link between Leptomitaceae and Rhipidiaceae is yet to be confirmed; more data are needed to justify such a conclusion. Presently, we may simply observe the interesting series of thallus forms within the traditional grouping, Leptomitales, with *Sapromyces* representing a putative, quasi-intermediate form. In any event, this is a group of organisms that invites further comparative morphological and developmental study—in addition, of course, to further ultrastructural and molecular investigations.

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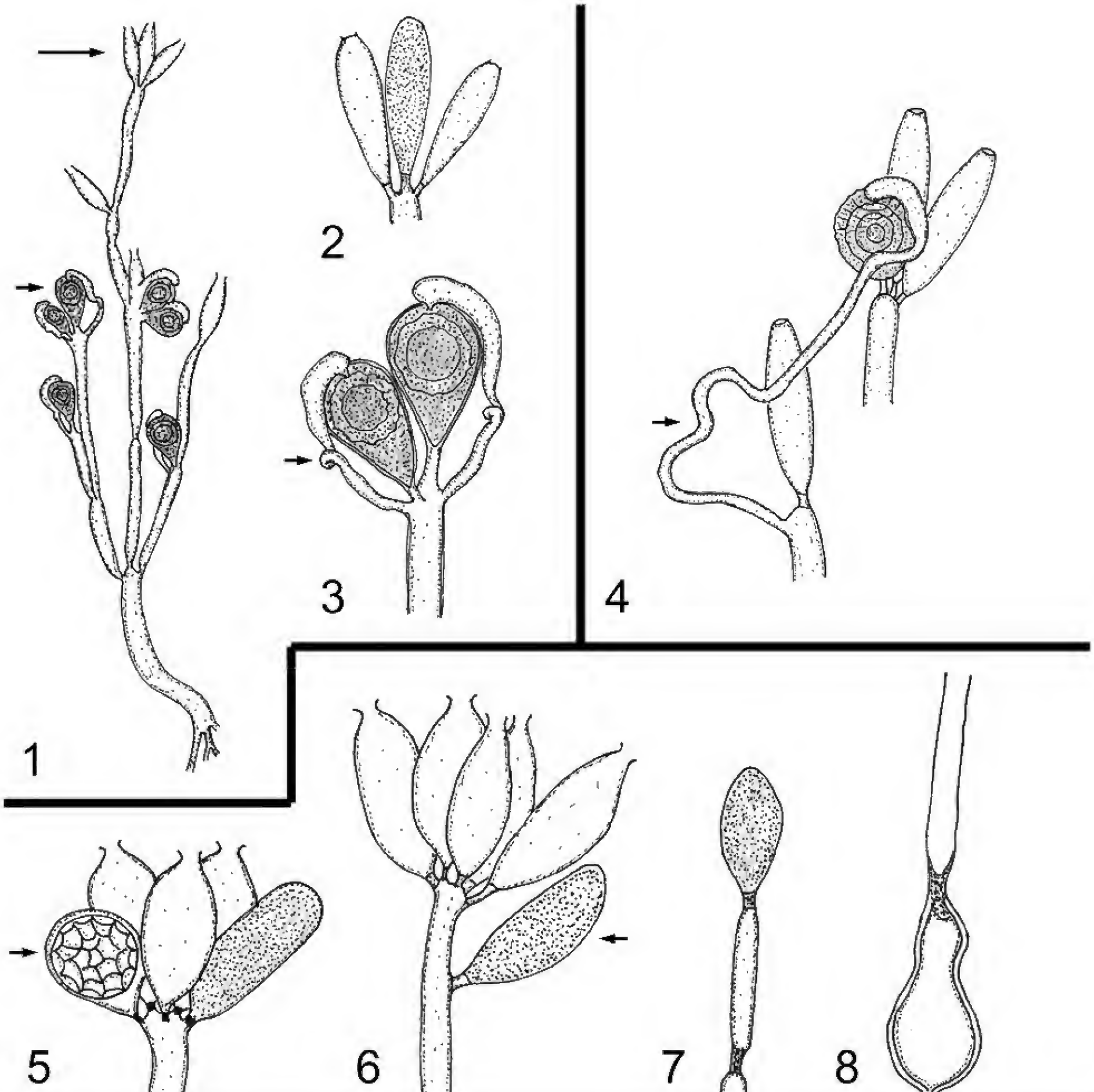
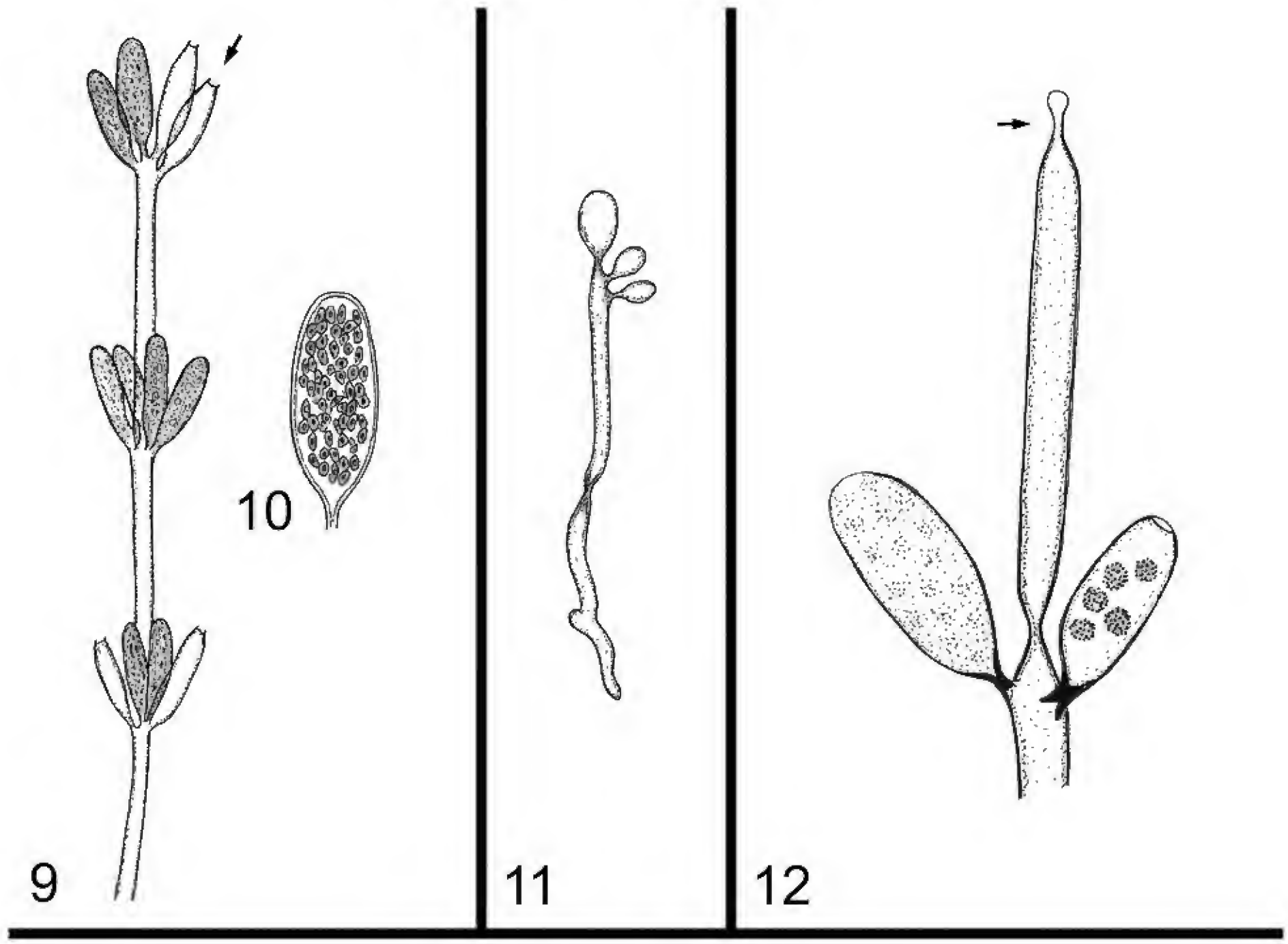
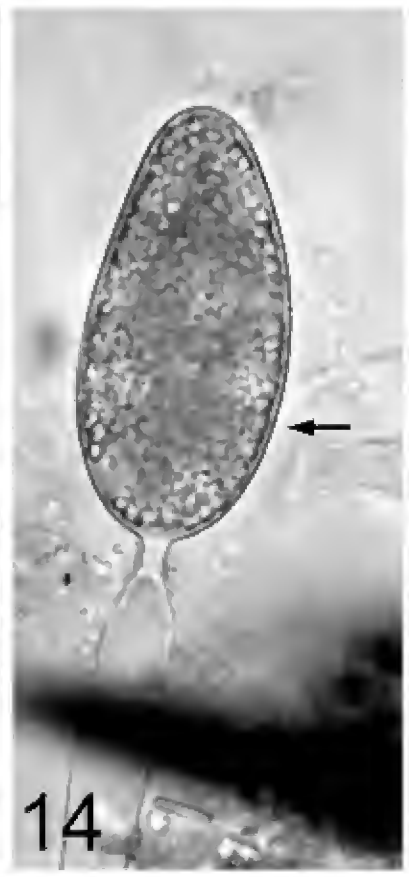
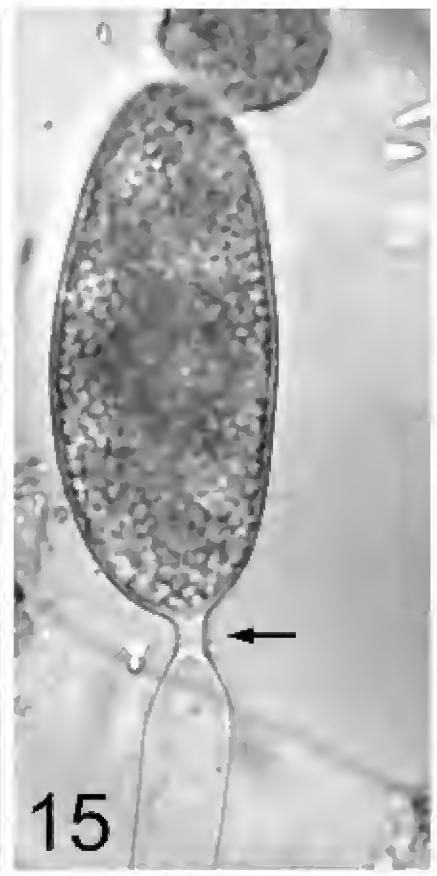


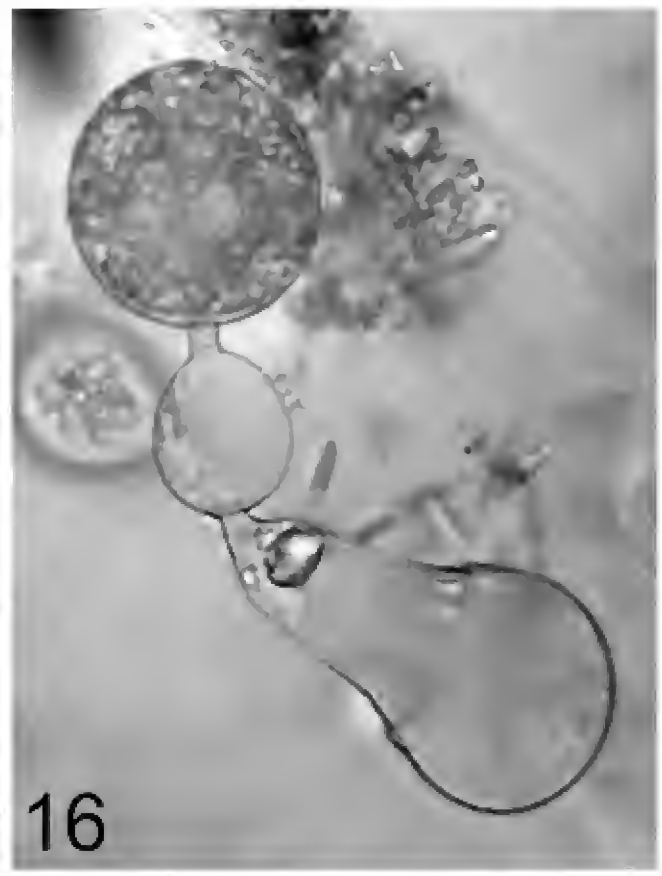
Figs. 1-3: *Sapromyces androgynus* (after Thaxter, 1896): Fig. 1, thallus with sex organs (short arrow), and (mostly emptied) sporangia (long arrow); basal pseudo-cell of thallus relatively undifferentiated, but branched ("holdfast-like") at its base. Fig. 2, "pedicelled" sporangia. Fig. 3, two oogonia and attached antheridia on same parent branch; note characteristic twist (arrow) of antheridial "stalk." **Fig. 4**: *Sapromyces elongatus* (after Sparrow, 1960): antheridium arising from different hyphal branch than oogonium; antheridial "stalk" (arrow) long and winding. **Figs. 5-8**: *Sapromyces indicus* (after Iyengar et al., 1955): Fig. 5, sporangial cluster and an oogonium (arrow); oospore wall, in oogonium, reticulate. Fig. 6, sporangia terminal, but one (arrow) formed from lateral budding. Fig. 7, branch (with sporangium) the result of development by hyphal constriction and budding. Fig. 8, thick-walled, pear-shaped basal "cell."

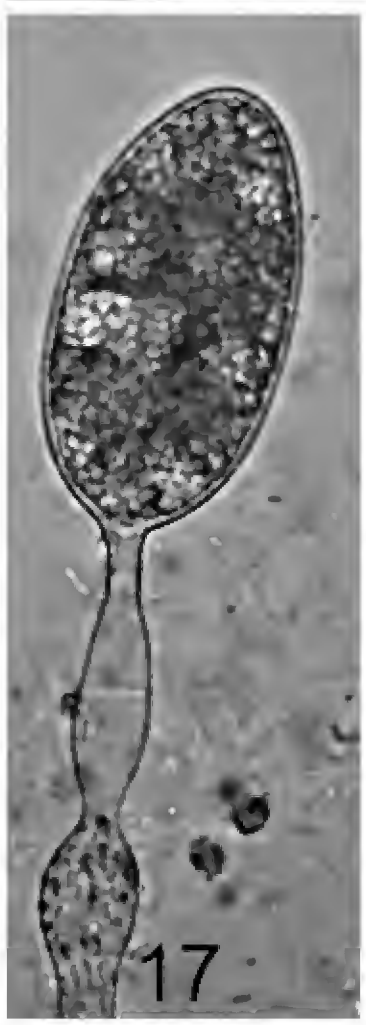


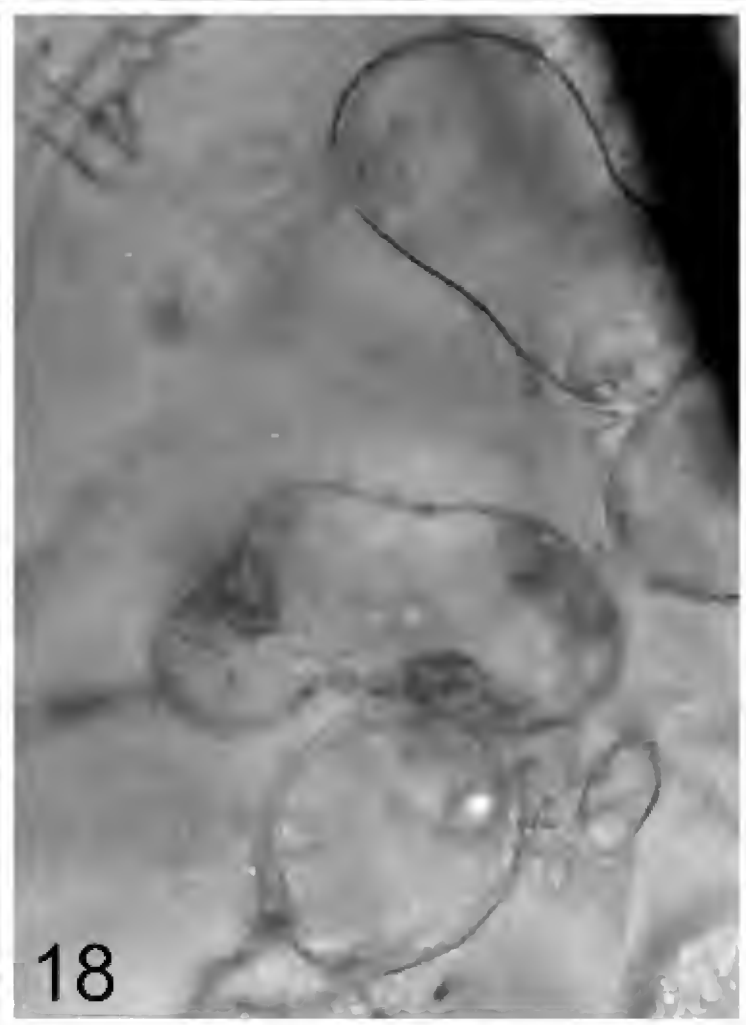
Figs. 9-10: "*Sapromyces reinschii*" (after Reinsch, 1878; this "species" probably = *S. elongatus*; however, based on only asexual material, the identity of *S. reinschii* has been difficult to confirm): Fig. 9, thallus segment with apparently "whorled" or "umbel-like" arrangements of zoosporangia; these clustered, often stalked ("pedicillate") sporangia arise by a simple budding process from a generally terminal (terminal at least at the point in time at which the sporangia arose) portion of a hyphal branch. Fig. 10, sporangium with incipient zoospores still contained within; zoospores will eventually be released from a generally circular, apical (initially papilla-like) opening in the apex of the sporangium; this apical "aperture" (see fig. 9, arrow) seems generally consistent in genus *Sapromyces* (compare, for example, fig. 9 with figs. 1, 4, 6 and 12). **Fig. 11**: "*Sapromyces dubius*" (after Reinsch, 1878), a doubtful species of *Sapromyces;* the exact taxonomic disposition of *S. dubius* remains uncertain (it was considered by Sparrow, 1960, to represent a fragment of a *Rhipidium* species, but this is likewise difficult to confirm). **Fig. 12**: Constriction/budding growth in *Sapromyces elongatus* (after Dick, 1973); see sub-terminal constriction (arrow), with small bud of protoplasm arising distally beyond (which will form the next hyphal pseudo-cell, or else a sporangium; compare with the resultant growth by budding in fig. 7, *S. indicus*).

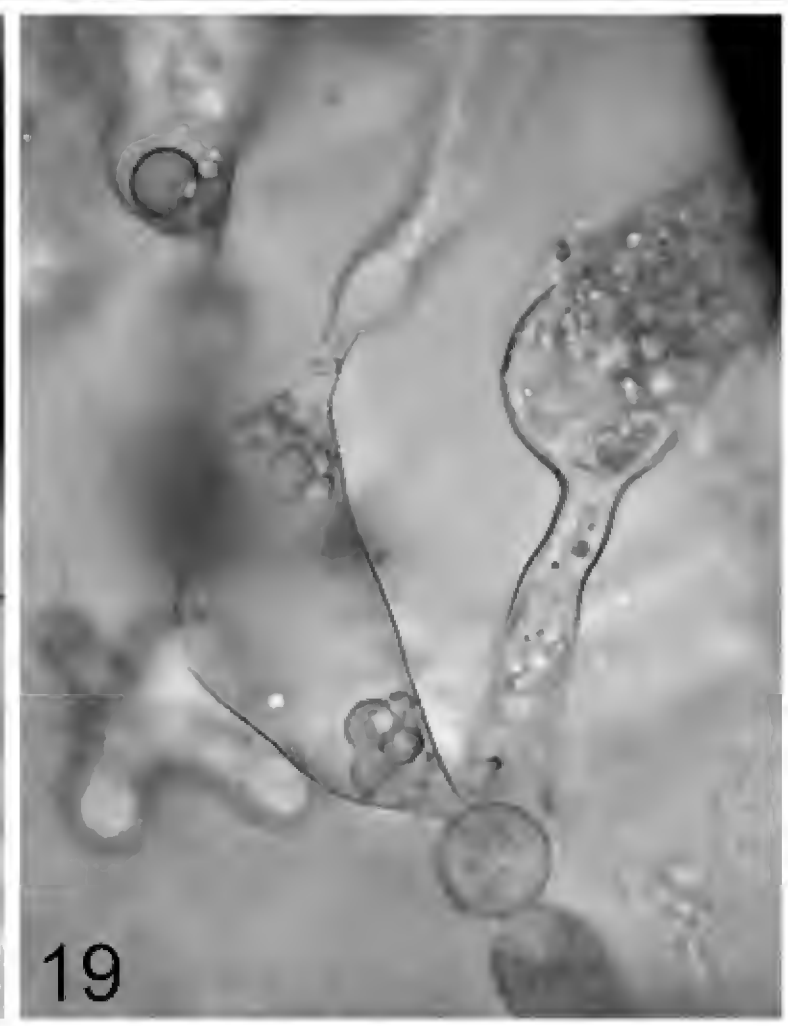












Figs. 13-15, 17: Representative photographs of generally apical sporangia, seen in our collection of *Sapromyces* (tentatively identified as *S. elongatus*); note characteristic, "young," ovate form of sporangium (e.g., Fig. 14, arrow), and absence of zoospore development, indicative of retention of juvenescent state (probably in response to depleted conditions of original habitat). Characteristic constriction (e.g., Fig. 15, arrow) beneath sporangium, results in "pedicelled" appearance; more or less solid area in constriction (Figs. 14, 15) constitutes "plug" of cellulin. Fig. 17, shows consequence of growth by successive constriction and budding (cf. figs. 7, 12), characteristic of *Sapromyces*; as seen (figs. 7, 17), this may result in production of a sporangium. **Figs. 16, 18, 19**: pseudo-cells of thallus may form unusual, irregular shapes; these may contain large, refractive granules (Fig. 19, bottom and top of cell occupying left-center of photo); such "cells" are possible "gemmae" (vegetative reproductive bodies), separating by thallus fragmentation (Fig. 18). Scale bar (in Fig. 13) = 20 μm for Figs. 13-19.

Figs. 20-25: Additional photographs of our specimens of *Sapromyces*, illustrating features of interest. Although sporangia were often seen to be solitary and terminal (figs. 14, 15), a number of branches of the thallus may exhibit one to several sporangia at or near the tip, these sometimes occurring in an apparently whorled pattern (Fig. 24, seen more or less in cross-section; see also fig. 9). When, for example, two sporangia are present, a sporangium may be terminal, and a second, lateral to it on the apex (see Figs. 20; 23, arrow; see also Fig. 13); or neither sporangium may quite attain an apical position (Fig. 21). Variation is evident in sporangial shape, from oblate-spheroid when young, to ovate, elliptic or obovate at a later stage (compare all sporangial photographs). Sporangial bases are often constricted, and may become plug-like (even darkened), cf. figs. 5, 12, 13 and 23. In addition to occurrence of sporangia at the sometimes almost "shouldered" apex of a hypha (see figs. 13, 20, 21), an oogonial initial can be observed to occur at the base of a sporangium; this oogonial initial (Fig. 22, arrow) may be distinguished by an almost spherical shape, relatively thin wall, and presence of two distinct regions within (the inner area can develop into ooplasm, the outer into periplasm). Fig. 25: branched thallus with constrictions; see small, terminal constriction and bud of protoplasm (arrow) by which growth occurs (compare with fig. 12). Scale bar (in Fig. 20) = 20 μm for Figs. 20, 21, 23, 24; bar in Fig. 22 = 20 μm; in Fig. 25 = 20 μm.

Geographic variation in the leaf essential oils of *Juniperus grandis* (Cupressaceae) III. San Gabriel Mtns. population.

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX, 76798, USA
email Robert_Adams@baylor.edu

and

Richard E. Riefner, Jr.
Research Associate, Rancho Santa Ana Botanic Garden, 1500 North College Avenue
Claremont, CA 91711-3157, USA.

ABSTRACT

Analysis of the volatile leaf oil of *J. grandis* from the San Gabriel Mtns. is presented along with analysis of trees from the San Bernardino Mtns. and the High Sierra, CA. The oil of *J. grandis*, San Gabriel Mtns., is dominated by sabinene (14.8%), δ-3-carene (13.4%) and α-pinene (10.6%) with moderate amounts of α-terpinene (4.9%), limonene (2.8%), β-phellandrene (4.2%), γ-terpinene (4.6%), terpinolene (3.8%), terpinen-4-ol (4.6%), bornyl acetate (1.5%), methyl geranate (1.6%), δ-cadinene (1.9%), elemicin (1.2%) and germacren-D-4-ol (1.6%). Its oil is very similar to that from the San Bernardino Mtns. The oils from San Gabriel and San Bernardino Mtns. were intermediate between *J. grandis* (High Sierra) and *J. osteosperma* (NV), suggesting the trees may be of hybrid origin. Published on-line **www.phytologia.org** *Phytologia 97(2): 94-102 (April 1, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus grandis (= J. occidentalis* var. *australis), J. occidentalis, J. osteosperma,* Cupressaceae, essential oils, terpenes, geographic variation.

Previously, Adams (2012) and Adams and Kauffmann (2010a) reported on geographic variation in the leaf essential oils of *J. grandis* R. P. Adams (= *J. occidentalis* var. *australis* (Vasek) A. & N. Holmgren). They found that the leaf oils of *J. grandis* contained two chemical races: High Sierra populations with oils dominated by δ-3-carene (17.9-30.0%) and low in sabinene, and the San Bernardino Mtns. population with oil low in δ-3-carene, but very high in sabinene (24.3%). In addition, they found the leaf oil of putative '*J. grandis*' of the Yolla Bolly Mtns. to be more similar to the oil of *J. occidentalis* than to that of *J. grandis*. Subsequent DNA sequencing gave support that the Yolla Bolly Mtns. juniper is a divergent form of *J. occidentalis* (Adams and Kauffmann, 2010b; Adams, 2014).

Adams (2012) analyzed the leaf oils of additional populations from Beckwourth and Stampede Meadows, CA. He found the oils from Stampede Meadows to be like those of *J. grandis* (Fig. 1), but the Beckwourth trees appear to be hybrids between *J. grandis* and *J. occidentalis* (Fig. 1).

The overall trend in the leaf oils of *J. grandis* shows the highest similarity of oils is in the High Sierra (Meyers and Sonora Junction) followed by Donner Pass, Stampede Meadows and the 9 Mile locality (Fig. 2). The San Bernardino Mtns. oils are much less similar and is actually more like the Yolla Bolly (*J. occidentalis*) oils than the nearby 9 Mile population.

In the present study, we report on the oil from a disjunct population of *J. grandis* from the San Gabriel Mtns., CA.

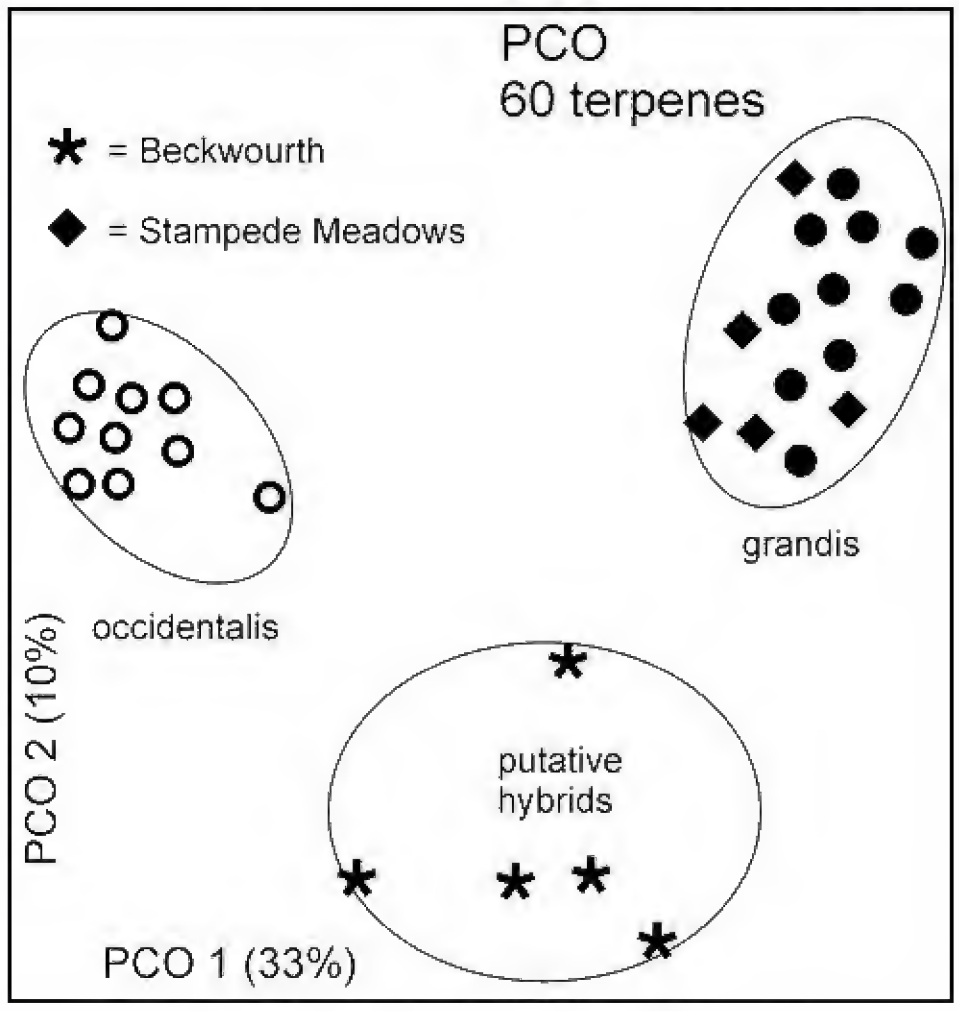


Figure 1. PCO based on 60 terpenes, ordinating *J. grandis, J. occidentalis* and putative hybrids from Beckwourth. From Adams (2012).

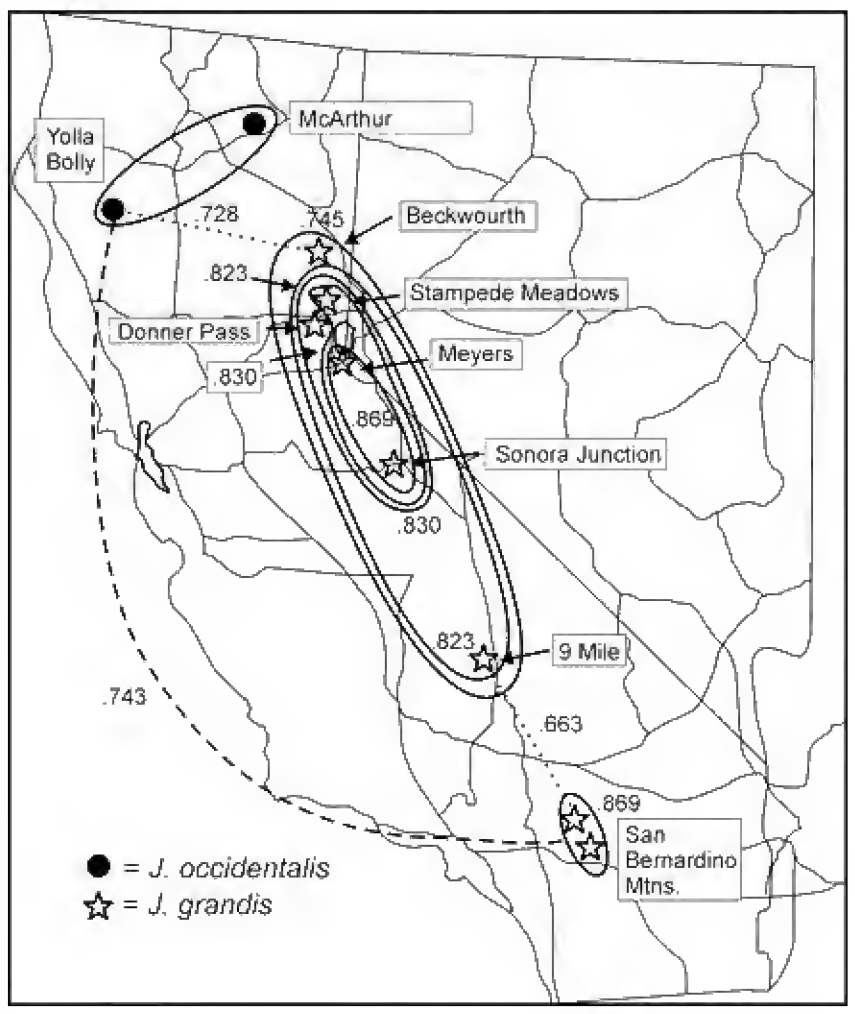


Figure 2. Contoured clustering based on *63* terpenoids. The dashed and dotted lines show unusual links. The numbers next the lines are the similarities. From Adams (2012).

MATERIALS AND METHODS

Plant material (see also Fig. 3): *J. grandis, Adams 11963-11967*, Jct. US 50 & CA 89, 38º 51.086N, 120º 01.244'W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA Hwy. 108, 38º 18.289'N, 111º 35.598'W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35º 54.003'N, 118º 02.078'W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34º 17.533'N, 116º 49.153'W, 2053 m, San Bernardino Co., CA; *Adams 12319-12322,* Onyx Summit on CA 38, 34° 11.524'N; 116° 43.227' W.2600 m, San Bernardino Co., CA; *Adams 12328-12331, 12367,* Donner Pass Summit on old US50, 39° 18.999' N; 120° 19.581' W. 2180 m, Placer Co., CA; *Adams 12332-12336,* on Stampede Meadows Rd. (Co. Rd 894 A1t), 5 mi. n of I80. 39° 24.966' N; 120° 05.249' W, 1660 m, Nevada Co., CA; *Adams 12337-12341,* 4.7 mi. n of Beckwourth on Beckwourth-Genesee Rd., 39°52.433'N; 120° 24.345'W, 1770 m, Plumas Co., CA; Lab acc. *Adams 13532-13536*, ex *Rick Riefner, Jr. 12-332, 12-335, 12-342-344*, clustered weathered trees on granitic rocks, uncommon, w of Mt. Baldy Notch on Devils Backbone Trail, San Gabriel Mtns., 6 - 7 m trees, multiple coalescing trunks, 34° 17' 9.595" N, 117° 37' 58.801"W, 2719-2891 m, 26 Aug 2012, Los Angeles Co., CA.

J. occidentalis, Adams 11940-11942, 12 km e of Jct. WA 14 & US 97 on WA 14, 45º 44.392'N, 120º 41.207'W, 170 m, Klickitat Co.; WA, *Adams 11943-11945*, 2 km s of Jct. US 97 & US 197 on US 97, 38

km ne of Madras, OR; 44° 53.676'N, 120° 56.131'W, 951 m, Wasco Co., OR; *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44° 02.390'N, 121° 20.054'W, 1132 m, Deschutes Co., OR; *Adams 11949-11951,* 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922'N, 120° 59.187'W, 1274 m, Deschutes Co., OR; *Adams 11952-11954,* 14 km e of Jct. OR66 & I5, on OR66, 42° 08.044'N, 122° 34.130'W, 701 m, Jackson Co., OR; *Adams 11957-11959*, on CA299, 10 km e of McArthur, CA, 41° 05.313'N, 121° 18.921'W, 1091 m, Lassen Co., CA; *Adams 11995-11998 (Kauffmann A1-A3, B1),* Yolla Bolly-Middle Eel Wilderness, 40° 06' 34"N, 122° 57' 59W, 1815- 2000 m, Trinity Co., CA, *Adams 12342-12346*, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178'N, 120° 50.211' W, 1570 m, Lassen Co., CA, *Adams 12347-12351*, on US 395, 5 km n of Madeline, 41° 05.867'N, 120° 28.456' W, 1695 m, Lassen Co., CA.

J. osteosperma, Hancock Summit, mile 38 on US 375, 37° 26.404'N, 115° 22.703'W, 1675 m, Lincoln Co. NV; *Adams 11125-11127*, McKinney Tanks Summit on US 6, 38° 07.005'N, 116° 54.103'W, 1933 m, Nye Co., NV. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

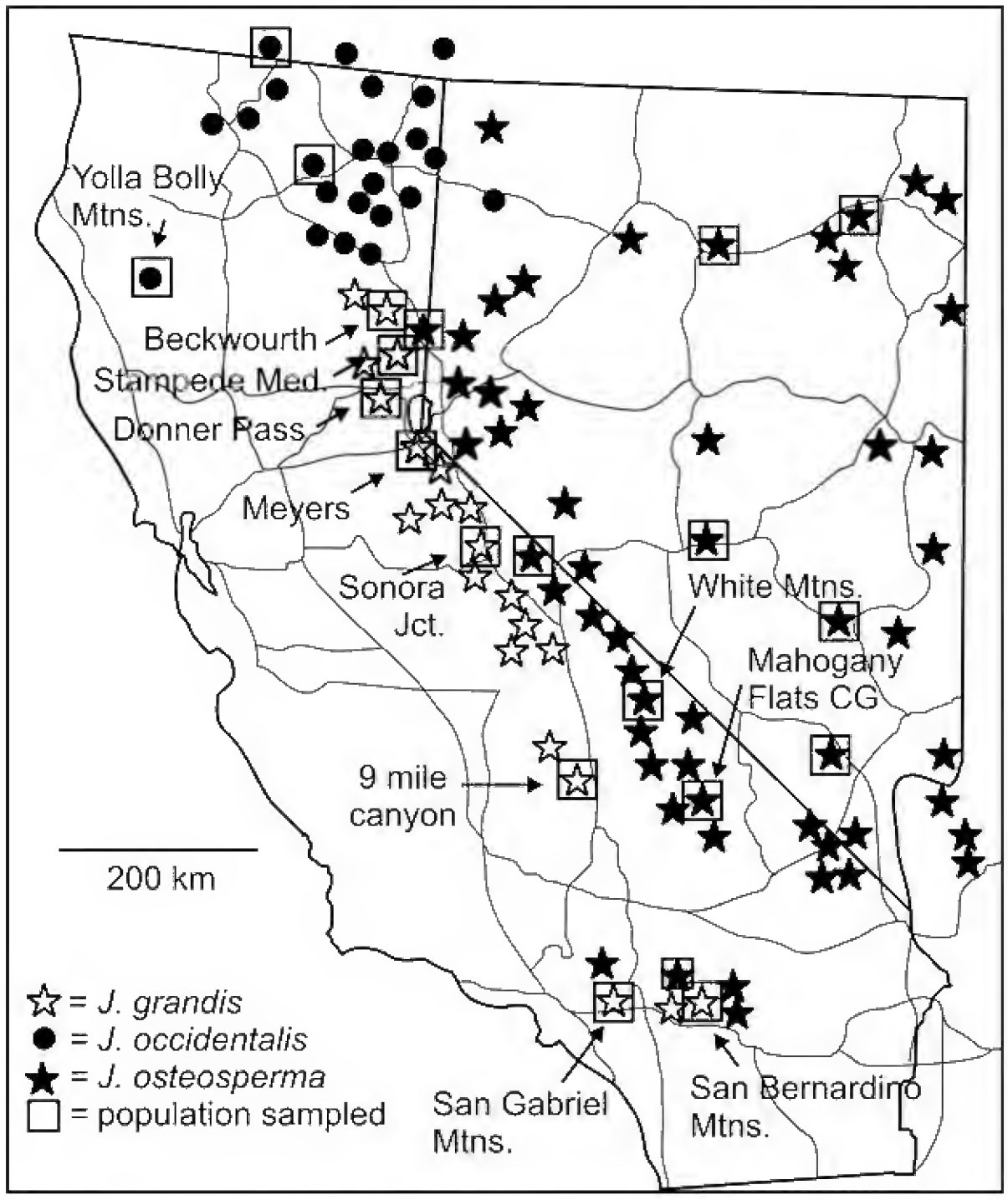


Figure 3. Distributions of *J. grandis*, *J. occidentalis* and *J. osteosperma*, modified from Vasek (1966) and Adams and Kaufmann (2010a). Note the San Gabriel Mtns., population; the focus of this study.

Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields. Oils were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The oil of *J. grandis*, San Gabriel Mtns., is dominated by sabinene (14.8%), δ-3-carene (13.4%) and α-pinene (10.6%) with moderate amounts of α-terpinene (4.9%), limonene (2.8%), β-phellandrene (4.2%), γ-terpinene (4.6%), terpinolene (3.8%), terpinen-4-ol (4.6%), bornyl acetate (1.5%), methyl geranate (1.6%), δ-cadinene (1.9%), elemicin (1.2%) and germacren-D-4-ol (1.6%). Its oil is very similar to that from the San Bernardino Mtns. (Table 1).

PCO (Principal Coordinates Ordination) based on 62 terpenes, using population averages resulted in three eigenroots before they appeared to asymptote accounting for 34.16%, 15.10% and 10.27% of the variation among OTUs. This ordination shows (Fig. 4) clear grouping of *J. osteosperma* (NV), *J. occidentalis* (n CA), *J. grandis* (High Sierra, CA), the Beckwourth population (*J. grandis* x occidentalis), and the southern *J. grandis* group (San Gabriel and San Bernardino Mtns.), which appear intermediate between *J. grandis* and *J. osteosperma* in the ordination (Fig. 4).

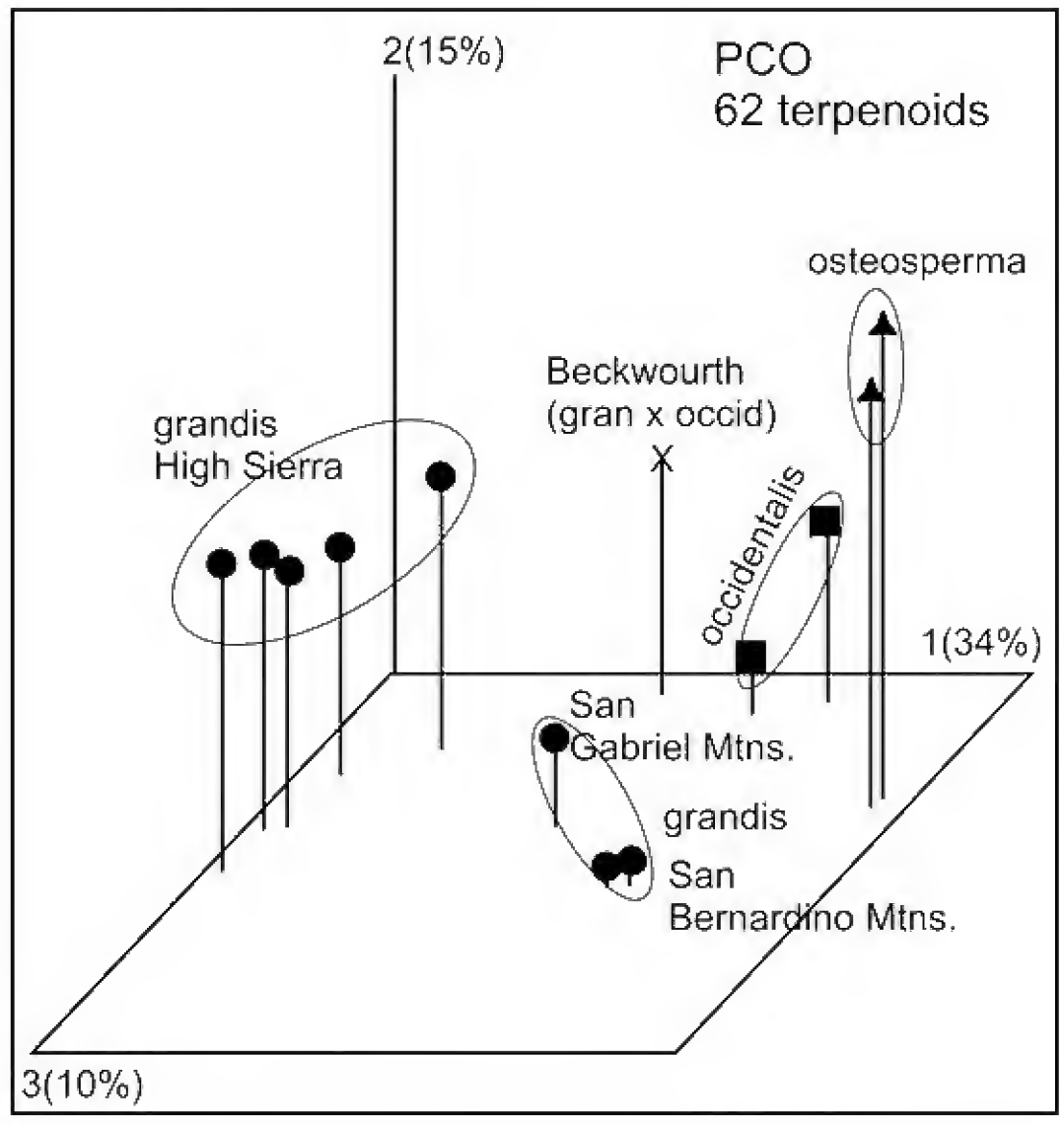


Figure 4. PCO based on 62 terpenoids, weights = 1. In this ordination, the San Gabriel and San Bernardino Mtns. populations appear to be intermediate in their oils.

To further examine the oils of *J. grandis,* individuals in southern California, ANOVA was performed between ten *J. grandis* oils from the High Sierra and ten *J. osteosperma* trees from Nevada. The resulting F ratios were used as character weights (F-1 weighting) for 40 terpenoids with the largest F values. Adams and Tsumura (2012) analyzed artificial hybrids between cultivars of

Cryptomeria japonica and found that F-1 weighting in PCO was the most effective method to discriminate between parents and hybrids. Later, Adams and Stoehr (2013) extended the study of the inheritance of terpenoids by examining *Pseudotsuga menziesii* F_1 hybrids (vars. *menziesii* X *glauca*). They found, again, that F-1 weighting in PCO was the most effective method to discriminate between parents and hybrids, confirming the earlier study by Adams (1982). PCO resulted in only 3 eigenroots that accounted 47%, 16% and 6% of the variation among OTUS. Figure 5 shows PCO between individuals from pure populations of *J. grandis* (High Sierra), *J. osteosperma* (Nevada) and individuals from the San Bernardino and San Gabriel Mtns. Notice that the reference individuals of *J. grandis* and *J. osteosperma* are in tight groups due to the F-1 weighting. The individuals from the San Bernardino and San Gabriel Mtns. are very loosely ordinated between *J. grandis* and *J. osteosperma*. There is some hint of differentiation between San Bernardino and San Gabriel Mtns. individuals (as indicated by the dashed line in Fig. 5).

The variation among the southern California *J. grandis* trees (Fig. 5) is seen in the composition in Table 2. Several compounds in trees are transgressive to the means (α-pinene, sabinene, δ-3-carene, α-terpinene, terpinen-4-ol, methyl geranate and elemicin) and exhibit extreme variation.

The loose grouping of the San Bernardino and San Gabriel Mtns. trees is suggestive of long term inter-crossing of hybrids and backcrosses, such that the populations are very diverse. It may be that if hybridization has occurred, it was during the Wisconsin glacial maximum when the ranges of *J. grandis* and *J. osteosperma* were highly overlapping. *Juniperus osteosperma* still grows at lower elevations in the basin below Big Bear Lake, while *J. grandis* grows at Big Bear and higher elevations, such that the taxa are scarcely, isolated. Alternatively, this may be a case of stabilized hybridization.

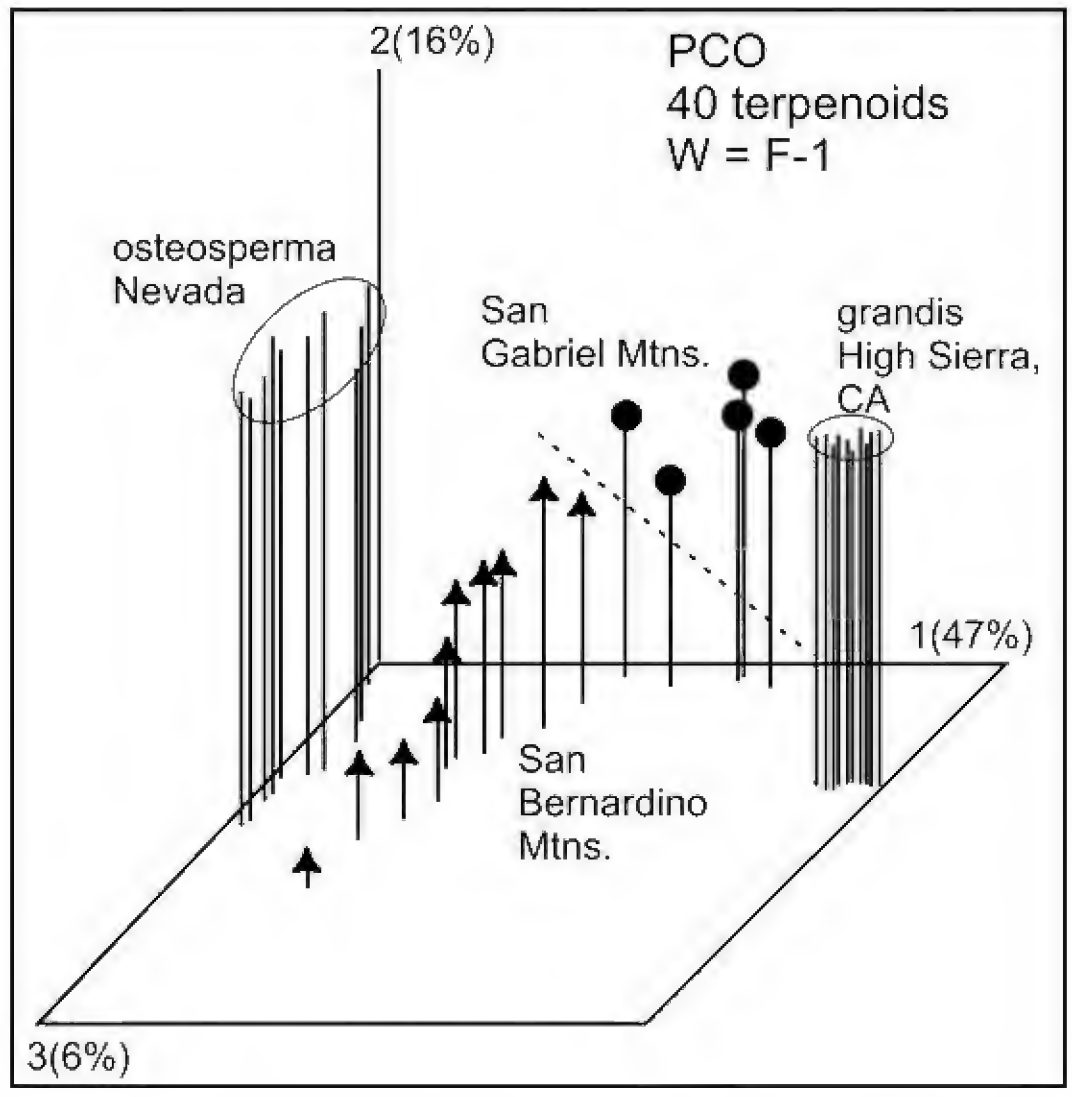


Figure 5. PCO based on 40, F-1 weighted terpenoids. The dashed line divides San Gabriel and San Bernardino Mtns. individuals.

It should also be noted that Adams and Kauffmann (2010b) found differences in trnS-trnG sequences between *J. grandis* (Meyers, CA, High Sierra) and *J. osteosperma* (Utah) but not between *J. grandis* (Big Bear, CA) and *J. osteosperma* (Utah). In addition, neither petN-psbM, nor trnD-trnT differed between *J. grandis* (Big Bear, CA) and *J. osteosperma* (Utah), suggestive that the *J. grandis* at Big Bear may contain the *J. osteosperma* cp genome. *Juniperus grandis* from both the San Bernardino and San Gabriel Mtns. have cinnamon colored bark and broad tapered trunks in older trees that are typical of *J. grandis* from the High Sierra. Additional research into the status of *J. grandis* in southern California is planned (RPA).

ACKNOWLEDGEMENTS

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Table 1. Leaf essential oil compositions for three populations of *J. grandis*, (Meyers, CA; Stampede Meadows, CA, Beckwourth, CA, Big Bear City, San Bernardino Mtns., CA and San Gabriel Mtns., CA) plus *J. osteosperma* (McKinney Tanks Summit, NV), and *J. occidentalis* (McArthur, CA). Compounds in boldface appear to separate taxa and were used in numerical analyses. KI = Kovats Index (linear) on DB-5 column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.

KI	Compound	grandis	grandis	grandis	grandis	grandis	osteo	occid
		San Gab	Big Bear	Meyers	Stamp	Beckw.	Nevada	McArt.
921	tricyclene	t	0.3	-	-	0.1	0.9	1.1
924	**α-thujene**	**0.8**	**2.3**	**-**	**-**	**0.4**	**0.5**	**1.0**
932	α-pinene	10.6	7.1	14.0	6.8	2.5	4.4	5.0
945	**α-fenchene**	**0.7**	**0.2**	**1.5**	**1.2**	**t**	**-**	**t**
946	camphene	0.8	0.3	-	-	0.3	1.1	1.0
953	thuja-2,4-diene	-	-	t	-	-	t	t
961	**verbenene**	**1.0**	**0.3**	**2.9**	**2.0**	**0.6**	**-**	**-**
969	**sabinene**	**14.8**	**24.3**	**-**	**t**	**10.7**	**10.2**	**12.0**
974	β-pinene	0.8	0.5	1.3	0.8	0.6	0.2	0.4
988	myrcene	0.6	1.7	3.1	3.4	3.5	1.7	1.3
1001	δ-2-carene	0.7	0.1	1.1	0.7	0.8	-	t
1002	α-phellandrene	1.2	0.4	1.6	1.6	4.0	0.3	0.8
1008	**δ-3-carene**	**13.4**	**2.8**	**27.3**	**17.0**	**1.6**	**-**	**1.0**
1014	α-terpinene	4.9	3.0	0.4	0.3	1.1	1.3	1.7
1020	p-cymene	1.7	6.5	1.4	0.2	2.0	2.4	10.7
1024	**limonene**	**2.8**	**1.6**	**1.2**	**t**	**t**	**2.1**	**0.9**
1025	**β-phellandrene**	**4.2**	**1.5**	**10.6**	**16.1**	**20.1**	**3.2**	**3.5**
1044	(E)-β-ocimene	t	0.3	t	t	0.2	t	0.1
1054	γ-terpinene	4.6	4.9	0.3	0.6	1.6	2.1	3.0
1065	**cis-sabinene hydrate**	**0.5**	**1.9**	**-**	**-**	**0.5**	**0.8**	**0.9**
1086	terpinolene	3.8	1.9	3.7	2.3	1.7	1.4	1.3
1092	96, 109,43,152, C10-OH	0.3	t	0.9	1.2	-	-	-
1095	**trans-sabinene hydrate**	**0.7**	**1.8**	**-**	**-**	**0.4**	**1.0**	**0.7**
1095	linalool	-	-	t	0.7	0.5	t	0.5
1100	55,83,110,156, unknown	-	-	-	-	-	-	0.3
1112	trans-thujone	t	0.2	-	-	-	-	t
1118	cis-p-menth-2-en-1-ol	0.7	0.7	0.8	1.7	2.7	0.6	0.7
1122	α-campholenal	-	-	t	-	-	0.3	-
1132	cis-limonene oxide (furanoid)	t	t	t	-	-	-	-
1136	trans-p-menth-2-en-1-ol	0.5	0.8	0.9	1.4	2.0	-	0.9
1141	**camphor**	**0.1**	**1.2**	**-**	**0.5**	**0.3**	**23.7**	**2.5**
1144	**neo-isopulegol**	**-**	**-**	**0.5**	**0.5**	**-**	**-**	**-**
1145	camphene hydrate	0.1	0.2	t	t	0.2	1.5	0.2
1154	**p-menth-1,5-dien-8-ol isomer**	**0.4**	**t**	**0.6**	**0.8**	**t**	**-**	**-**
1154	sabina ketone	t	0.9	-	-	t	0.8	0.4
1161	p-menth-1,5-dien-8-ol	0.4	t	0.3	-	-	-	-
1165	**borneol**	**t**	**0.1**	**-**	**-**	**-**	**6.0**	**2.2**
1166	coahuilensol	0.5	t	t	0.7	1.2	t	0.6
1174	**terpinen-4-ol**	**4.6**	**9.3**	**0.4**	**0.8**	**3.7**	**8.3**	**6.7**
1176	**m-cymen-9-ol**	**-**	**-**	**0.4**	**1.2**	**0.4**	**-**	**-**
1176	cryptone	-	-	-	-	t	-	-
1179	p-cymen-8-ol	0.2	1.0	0.4	1.1	0.2	0.5	0.5
1186	α-terpineol	0.4	0.3	1.2	0.3	-	0.4	0.4
1195	myrtenol	0.3	0.2	-	-	-	0.2	-
1195	cis-piperitol	0.3	0.2	0.4	0.6	0.6	0.3	0.2
1207	trans-piperitol	0.3	0.6	0.9	1.4	1.0	0.3	0.3
1219	coahuilensol, methyl ether	0.5	0.2	-	-	-	0.2	-

KI	Compound	grandis	grandis	grandis	grandis	grandis	osteo	occid
		San Gab	Big Bear	Meyers	Stamp	Beckw.	Nevada	McArt.
1223	**citronellol**	**0.6**	**0.2**	**t**	**0.4**	**0.4**	t	**8.4**
1230	**trans-chrysanthenyl acetate**	**0.7**	**0.4**	**3.9**	**3.2**	**0.5**	**-**	**-**
1238	cumin aldehyde	t	0.3	-	-	-	0.3	0.2
1239	carvone	-	-	t	0.3	-	0.6	-
1249	piperitone	0.6	t	1.2	2.0	0.9	t	0.2
1253	trans-sabinene hydrate acetate	t	0.6	-	-	-	-	-
1254	linalool acetate	-	-	-	-	-	-	0.1
1255	4Z-decenol	-	-	0.4	-	-	-	-
1257	methyl citronellate	0.5	0.1	0.2	0.2	-	-	-
1274	neo-isopulegyl acetate	-	-	0.3	0.2	-	-	-
1284	**bornyl acetate**	**1.5**	**2.2**	**0.4**	**0.8**	**5.2**	**16.6**	**9.5**
1285	**safrole**	**-**	**-**	**0.3**	**0.1**	**-**	t	**-**
1298	carvacrol	t	0.2	0.2	0.3	0.3	t	0.4
1298	3'-methoxy-acetophenone	0.4	0.2	-		-	-	-
1319	149,69,91,164, phenolic	-	-	0.8	-	-	-	-
1322	**methyl geranate**	**1.6**	**1.8**	**-**	**1.4**	**16.3**	-	**1.0**
1325	p-mentha-1,4-dien-7-ol	0.1	0.7	-	-	-	t	t
1332	cis-piperitol acetate	-	-	0.4	-	-	-	-
1343	trans-piperitol acetate	0.1	t	0.3	-	-	-	-
1345	α-cubebene	t	t	-	-	-	-	t
1374	α-copaene	0.1	0.2	-	-	t	-	1.0
1387	β-bourbonene	0.1	0.3	0.5	-	-	-	0.2
1388	**79,43,91,180, unknown**	**-**	**-**	**0.3**	**0.3**	**0.1**	**-**	**-**
1389	**111,81,151,182, unknown**	**0.5**	**0.4**	**1.0**	**1.2**	**0.2**	**-**	**-**
1403	methyl eugenol	-	-	t	0.2	-	-	-
1417	(E)-caryophyllene	t	0.2	-	-	-	-	-
1429	cis-thujopsene	-	-	-	-	-	-	0.9
1430	β-copaene	t	t	-	-	-	-	-
1448	cis-muurola-3,5-diene	0.1	0.2	t	-	-	-	-
1451	trans-muurola-3,5-diene	-	-	-	-	-	-	0.1
1452	α-humulene	t	t	-	-	-	-	-
1465	cis-muurola-4,5-diene	t	0.1	-	-	-	-	0.1
1468	pinchotene acetate	-	-	-	0.8	1.4	0.5	0.6
1471	**121,105,180,208,phenol**	**0.7**	**0.3**	**0.3**	**0.7**	**1.3**	-	**-**
1471	dauca-5,8-diene	0.1	0.2	-	-	-	-	-
1475	trans-cadina-1(6),4-diene	-	-	-	-	-	-	0.3
1478	**γ-muurolene**	**0.1**	**0.2**	**-**	**0.2**	**t**	-	**0.8**
1484	germacrene D	0.2	0.3	0.2	0.2	t	-	0.3
1491	43,207,161,222, C15-OH	0.4	0.3	-	-	-	-	-
1493	trans-muurola-4(14),5-diene	-	0.2	-	0.3	t	-	0.4
1493	epi-cubebol	0.4	0.5	-	0.4	0.2	t	0.4
1500	α-muurolene	-	-	0.3	0.3	t	t	1.1
1513	**γ-cadinene**	**0.4**	**1.2**	**1.3**	**0.6**	**0.3**	**t**	**3.7**
1518	endo-1-bourbonanol	1.1	1.5	0.4	1.2	0.4	-	0.4
1521	**trans-calamenene**	**t**	**2.3**	**-**	**0.6**	**t**	**-**	**-**
1522	δ-cadinene	1.9	t	1.1	0.7	0.8	0.2	4.1
1533	trans-cadina-1,4-diene	0.1	0.1	-	-	-	-	0.1
1537	α-cadinene	0.1	0.2	t	t	-	-	0.4
1544	α-calacorene	t	t	-	-	-	-	0.3
1548	elemol	0.5	0.9	-	0.1	0.7	0.9	-
1555	**elemicin**	**1.2**	**t**	**1.5**	**0.7**	**0.5**	**-**	**-**
1559	germacrene B	t	0.1	-	-	-	-	-
1561	(E)-nerolidol	-	-	-	-	t	-	-
1574	germacrene-D-4-ol	1.6	1.1	0.7	0.8	0.4	t	0.6
1582	caryophyllene oxide	t	0.3	t	t	-	t	-
1586	gleenol	-	-	-	-	-	-	0.3

KI	Compound	grandis	grandis	grandis	grandis	grandis	osteo	occid
		San Gab	Big Bear	Meyers	Stamp	Beckw.	Nevada	McArt.
1587	trans-muurol-5-en-4-α-ol	t	t	-	-	-	-	-
1607	β-oplopenone	0.6	0.8	0.4	0.4	0.1	t	0.4
1618	1,10-di-epi-cubenol	t	t	t	-	-	-	0.2
1627	1-epi-cubenol	0.4	0.5	t	0.7	0.3	-	1.6
1630	γ-eudesmol	t	t	-	-	t	0.2	-
1638	epi-α-cadinol	0.6	0.6	0.7	0.6	0.3	t	1.1
1638	epi-α-muurolol	0.7	0.6	0.7	0.6	0.4	t	1.2
1644	α-muurolol	0.2	0.1	t	0.2	t	t	0.7
1649	β-eudesmol	0.1	0.2	0.4	t	0.2	0.2	-
1652	α-eudesmol	0.8	0.6	-	-	0.2	0.2	-
1652	α-cadinol	0.8	0.7	1.6	1.3	0.9	0.2	1.8
1675	cadalene	t	0.1	-	-	-	-	0.3
1687	43,167,81,238, unknown	0.1	0.3	-	-	-	-	-
1688	shyobunol	-	-	0.2	t	-	-	-
1739	oplopanone	0.1	0.2	t	0.2	-	t	-
1987	**manoyl oxide**	**t**	**t**	**t**	**-**	**-**	**-**	**3.2**
2009	epi-13-manoyl oxide	-	-	-	-	-	-	t
2056	manool	-	-	t	0.4	-	-	-
2055	abietatriene	-	-	t	t	-	-	-
2298	4-epi-abietal	-	-	t	0.2	-	-	-
2312	abieta-7,13-dien-3-one	-	-	-	-	-	0.2	-

Table 2. Variation in major oil components for several *J. grandis* individuals from the San Bernardino and San Gabriel Mtns. Column heading is tree # followed by SB (San Bernardino) or SG (San Gabriel Mtns.). San Gab and San Bern are population averages for San Gabriel and San Bernardino Mtns. populations. Some compounds with extreme variation are in boldface.

KI	Compounds	11993SB	13533SG	12321SB	12319SB	13535SG	San Gab	San Bern
932	**α-pinene**	**4.0**	**4.9**	**9.6**	**5.1**	**4.4**	**10.6**	**7.1**
961	verbenene	0.2	0.5	0.5	1.2	1.4	1.0	0.3
969	**sabinene**	**24.6**	**24.4**	**17.3**	**13.8**	**13.5**	**14.8**	**24.3**
974	β-pinene	0.3	0.3	0.9	0.4	0.3	0.8	0.5
988	myrcene	1.9	2.7	1.2	1.7	1.9	0.6	1.7
1002	α-phellandrene	0.2	1.3	0.2	0.4	0.4	1.2	0.4
1008	**δ-3-carene**	**0.3**	**0.4**	**0.8**	**11.5**	**17.3**	**13.4**	**2.8**
1014	**α-terpinene**	**3.5**	**6.6**	**2.9**	**2.6**	**0.1**	**4.9**	**3.0**
1024	limonene	0.8	3.2	0.3	0.6	1.2	2.8	1.6
1025	β-phellandrene	0.9	5.0	1.3	2.4	2.0	4.2	1.5
1054	γ-terpinene	5.8	5.6	5.3	4.7	3.0	4.6	4.9
1065	cis-sabinene hydrate	2.6	0.9	1.6	1.0	0.5	0.5	1.9
1086	terpinolene	1.9	3.5	1.7	2.7	2.8	3.8	1.9
1095	trans-sabinene hydrate	2.5	0.9	2.0	1.6	0.5	0.7	1.8
1174	**terpinen-4-ol**	**11.7**	**6.5**	**13.2**	**10.6**	**5.9**	**4.6**	**9.3**
1223	citronellol	t	0.4	0.3	0.1	1.8	0.6	0.2
1284	bornyl acetate	0.2	0.5	0.4	0.1	0.2	1.5	2.2
1322	**methyl geranate**	**0.8**	**1.8**	**0.9**	**0.6**	**4.6**	**1.6**	**1.8**
1513	γ-cadinene	1.2	0.3	1.1	1.6	0.3	0.4	1.2
1518	endo-1-bourbonanol	1.4	1.8	2.6	2.9	0.6	1.1	1.5
1522	δ-cadinene	1.8	2.3	2.2	2.4	1.3	1.9	t
1555	**elemicin**	**t**	**0.1**	**t**	**t**	**6.5**	**1.2**	**t**
1574	germacrene-D-4-ol	1.0	1.6	1.5	1.7	1.0	1.6	1.1
1607	β-oplopenone	0.8	0.5	1.1	1.4	0.9	0.6	0.8
1638	epi-α-cadinol	0.7	0.6	0.6	0.9	0.7	0.6	0.6
1652	α-eudesmol	0.6	0.8	0.6	1.0	1.1	0.8	0.6
1652	α-cadinol	0.7	0.8	0.7	1.1	1.2	0.8	0.7

Photosynthetic characteristics of *Garrya ovata* Benth. (Lindheimer's silktassle, Garryaceae) at ambient and elevated levels of light, CO_2 and temperature

Matthew B. Grunstra
Department of Ecology and Environmental Science, University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249, USA
Matthew.Grunstra@utsa.edu

and

O. W. Van Auken
Department of Biology, University of Texas at San Antonio, One UTSA Circle, San Antonio, TX 78249, USA
oscar.vanauken@utsa.edu

ABSTRACT

Garrya ovata Benth. (Lindheimer's silktassle) is a shrub or small tree found in woodlands and savannas of the central Texas Edwards Plateau region. Plant communities where *G. ovata* occurs are mainly *Juniperus ashei/Quercus virginiana* woodlands or savannas with intercanopy grassland gaps or patches. These communities are found in stressful environments of shallow soils, high summer temperatures, and inconsistent low rainfall. Currently, *G. ovata* is a low density, woody understory species in these communities. Although *G. ovata* was only found in the understory, this study suggests it is more of a sun or edge species and not truly a shade or understory species. Maximum photosynthetic rate (A_{max}), dark respiration (R_d), intercellular CO_2, light saturation (L_{sp}) and water use efficiency significantly increased when light levels and CO_2 concentrations were elevated but not when temperatures were elevated. Stomatal conductance decreased when the CO_2 concentration doubled but was little affected by elevated temperature. These findings suggest that *G. ovata* should be more common in central Texas *Juniperus/Quercus* woodlands or savannas as well as in associated gaps or grasslands today and imply that it will have a higher density in these communities in a future high CO_2 environment. However, they do not explain why it has a low density in these communities today. Published on-line **www.phytologia.org** *Phytologia 97(2): 103-119 (April 1, 2015).* ISSN 030319430.

KEY WORDS: elevated CO_2, elevated temperature, light response, gas exchange, photosynthesis, respiration, water use efficiency

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Today the major canopy species in central Texas woodlands and savannas are *Juniperus ashei* Buchh. and *Quercus virginiana* Mill. (Ash juniper and hill country live oak)(Van Auken et al. 1981; Van Auken 1988; Van Auken and McKinley 2008). The potential composition and structure of these communities in a predicted future high CO_2 world is unknown. There are various understory woody species in these communities that could be canopy species today that may become canopy species in the future including *Garrya ovata* Benth. (Lindheimer's silktassle), *Diospyros texana* Scheele (Texas persimmon), *Rhus virens* Gray (evergreen sumac), *Sophora secundiflora* (Ort.) DC (Texas mountain laurel), several species of Quercus (L., oaks) and a few other Leguminosae (Juss., legumes)(Van Auken et al. 1981; Van Auken 1988; Van Auken and McKinley 2008). Most of these understory species including *Garrya ovata* have received little ecological or environmental study.

Juniperus woodlands occur in many parts of the northern hemisphere varying in elevation, climate and species composition (Wells 1965; Gould 1969; Blackburn and Tueller 1970; Correll and Johnston 1970; Little 1971; Baskin and Baskin 1986, 1988; West 1988; Dick-Pettie 1993; Van Auken and McKinley 2008). In North America they are found from the east coast through the Great Plains to the low

and mid-elevations of the mountains of the western United States, Canada and Mexico (Little 1971; Johnson and Alexander 1974; Elias 1980; Hora 1981;). In the past, in central and western North America, they occurred along canyon walls, steep slopes or other areas that were protected from fire (Bray 1904; Foster 1917; Smeins 1980).

Today, these *Juniperus* communities are extensive and have been treated as stable communities by most (Bray 1904; Gould 1969; Little 1971; Correll and Johnston 1970; Hora 1981; West 1988; Dick-Pettie 1993; Van Auken and McKinley 2008). However, various studies of encroachment and invasion have suggested that these are pioneer or intermediate communities that may lead to the development of forests or other types of woodlands (Jackson and Van Auken 1997; Van Auken et al. 2004; Van Auken and McKinley 2005). Over time, area covered and the species composition of these communities has apparently changed and will continue to transform, but their future composition and structure is unknown (Miller and Wigand 1994; Miller et al. 2000).

Changes in density, species composition and community distribution in the past 15-20,000 years have been due to climate warming and glacial retreat (NCA 2014). More recently, the past 200-300 years, changes have been attributed to the introduction of large populations of domestic ungulates causing high levels of constant grass herbivory which reduced the grass competitive ability and biomass and caused a decrease in fire frequency because of lack of fuel (Archer et al.1995; Bush and Van Auken 1995; Van Auken and Bush 1997; Van Auken 2000b). These more recent conditions of decreased grass biomass and fire frequency have led to the formation of *Juniperus/Quercus* savannas and woodlands. Other factors such as soil moisture, shading, and seed dispersal seem secondary (Breshears et al.1997, 1998; Martens et al. 2000, 2001; Joy and Young 2002; Wayne and Van Auken 2002, 2004). Conditions that may be more important for future community change include elevated atmospheric CO_2 concentration and temperature (NCA 2014).

Many *Juniperus* woodland communities including those in central Texas tend to be fairly open with gaps or open patches with shallow soils populated by grass and other herbaceous species (Quarterman 1950; Baskin and Baskin 1978, Quarterman et al.1993; Terletsky and Van Auken 1996; Baskin and Baskin 2000; Van Auken 2000a). Canopy density can be high and canopy cover is 40 to 90% (Van Auken et al.1981; Smeins and Merrill 1988; Van Auken and McKinley 2008). There are many very low density woody species in these communities but most are shrubby and it is unknown if some or most will or could become part of the woodland canopy in the future.

Many consider the entry of new woody species into these former grasslands, savannas or woodlands as encroachment or invasion (Van Auken 2000b; Van Auken and McKinley 2008; Van Auken 2009). However, it appears that community succession is the process governing replacement dynamics as this conversion from grassland to woodland continues. In the future, these *Juniperus/Quercus* woodlands may remain simple communities dominated by a few species or the *Juniperus* species may be replaced by other species from within or below the canopy or from elsewhere to progress to another successional stage or community type. This pattern is controlled by the interaction of the adult canopy species, surface light levels, soil moisture and nutrients, herbivory and fire (Jackson and Van Auken 1997; Batchelor and Fowler 2004; McKinley and Van Auken 2004; Van Auken et al. 2004; Van Auken and McKinley 2005). Due to modifications of these factors through time, the structure of many of these woodlands will probably change, but the direction of the changes and the future composition of the communities are unknown. Other environmental factors are changing and will continue to change in the future as well. These other factors include levels of atmospheric CO_2 and temperature which have been increasing and are expected to continue increasing into the foreseeable future (NCA. 2014).

We hypothesize that the *Juniperus/Quercus* communities in central Texas will change in the future. We further hypothesize that new understory species will be introduced and some of the current

understory species will become part of the canopy and may become dominant overstory or canopy species in the future. We hypothesize that elevated levels of atmospheric CO_2 and temperature will be the driving forces for these changes. In the present study, we have examined current *Juniperus/Quercus* community composition and structure specifically as it relates to *Garrya ovata.* We have also examined the short term response of *G. ovata* to ambient and elevated levels of light, CO_2 and temperature.

METHODS

Study sites were located in central Texas in Eisenhower Park, Lost Maples State Natural Area and a remote part of the University of Texas at San Antonio campus. Each area has slight variations in soil type, precipitation, temperature, plant communities previously observed, as well as length of time since last known exposure to grazing. Eisenhower Park is a 128 ha San Antonio city park in northern Bexar County, Texas (98°34'26"W, 29°37'19"N). Topography is rolling with slopes between 4.5° and 13.5° (Taylor et al.1962) and soils are clayey-skeletal, smectitic, thermic lithic calciustolls (USDA 2000) in the Tarrant association with surface horizons between 0 and 25 cm thick (Taylor et al.1966). The subsurface is heavily fractured limestone over limestone bedrock. Local climate is subtropical – subhumid (Arbingast et al.1976). Mean annual temperature for the area is 20 °C and ranges between monthly means of 9.6 °C in January and 29.4 °C in July (NOAA 2001). Mean annual precipitation is 78.7 cm and bimodal with peaks in May (10.7 cm) and September (8.7 cm) (NOAA 2001). Monthly precipitation is highly variable with usually very little reported in June and July. Eisenhower Park is a natural area with no domestic livestock for the past 55 years. The Park is composed of large areas of *Juniperus ashei/Quercus virginiana* woodlands or savannas on former grassland sites and is considered representative of similar communities found in this region (Van Auken et al.1981).

Lost Maples State Natural Area is in Bandera and Real counties, Texas (99°34'59"W, 29°49'11"N). The Park is 880 ha, located approximately 8 km north of Vanderpool, Texas on the Sabinal River with elevation from 550 to 686 m. Climate is similar to Eisenhower Park with a mean annual temperature of 20°C and mean annual precipitation of 89.1 cm with peaks in May and September (NOAA 2001). Uplands and slopes are dominated by similar *Juniperus/Quercus* savannas and woodlands. The third study site was located in a remote, undeveloped area on the west campus of the University of Texas at San Antonio. This area has similar topography, soils, climate and species as the other two sites.

Plant surveys and population estimations were completed to determine the community structure and population characteristics and to identify the woody species present at the Eisenhower and Lost Maples locations in both the canopy and understory. Mature relatively undisturbed *J. ashei/Q.virginiana* woodland communities were selected for study. Transects were established in each study area in a contiguous woodland with eight transects in Eisenhower City Park and three transects in Lost Maples State Natural Area.

Each transect consisted of contiguous, side by side, 5 by 5 m quadrats. Transects were 50 m in length for a total of 20 quadrats per transect. Sampling included all canopy trees and understory woody plants. Basal circumferences of all woody plants were measured at the soil surface and converted to basal areas. Total density, species density, total basal area and species basal area were calculated from these measurements. Sample adequacy was confirmed using density stabilization curves with all samples being adequate (Van Auken et al. 2005).

Gas exchange measurements were made at the University of Texas at San Antonio field site in the summer of 2007. Three *Garrya ovata* plants were selected for measurement of physiological responses at both ambient levels and elevated levels of CO_2 and temperature. Steady state photosynthetic light response curves (A_{net} vs. PAR) were completed (Van Auken and Bush 2009). Photosynthetic response

curves were measured on fully expanded leaves at mid-day (1000 – 1400 hours) when relative humidity had stabilized (Turner and Knapp 1996). One fully expanded leaf per plant served as a replicate and was placed into the cuvette of a portable photosynthetic meter (LICOR® LI-6400). Each leaf covered the entire chamber (2 x 3 cm). Measurements made and recorded for each plant were: A_{net} (net photosynthesis = μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), C_i (intercellular [CO_2] = μmol $CO_2 \cdot$mol air^{-1}), T_{leaf} (chamber leaf temperature = °C), T_{air} (air temperature outside the chamber = °C), PAR (photosynthetic active radiation = $\mu mol \cdot m^{-2} \cdot s^{-1}$), *g* (stomatal conductance = $mol \cdot H_2O \cdot m^{-2} \cdot s^{-1}$) and *E* (transpiration = $mmol \cdot H_2O \cdot m^{-2}\, s^{-1}$).

The leaf chamber was used to mimic varying degrees of environmental change. This was accomplished through systematic manipulations of the light level, CO_2 concentration, and temperature. The leaf chamber's relative humidity was maintained at 30 - 40% and the gas flow rate was set at 400 μmol s^{-1}. A stable coefficient of variation (< 1%) was obtained for each measurement before recording and moving to the next programmed setting. Light levels were begun at 1800 $\mu mol \cdot m^{-2}\, s^{-1}$ and decreased to 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, 75, 50, 25, 10, 5 and finally 0 $\mu mol \cdot m^{-2}\, s^{-1}$. Both photosynthetic light curves as well as CO_2 response curves were measured for several different combinations of the leaf chamber [CO_2] and temperature conditions.

The leaf chamber [CO_2] was first set at current ambient levels (390 $\mu L \cdot L^{-1}$). While maintaining ambient CO_2, a light curve was completed starting at 1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and moving downwards to 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ while maintaining a chamber temperature of 35°C. This temperature was chosen based on the mean high temperatures for San Antonio during the summer months of June, July and August. Light curves were repeated holding the ambient [CO_2] constant while raising the chamber temperature to 40°C and then to 45°C.

The leaf chamber CO_2 was then raised to 1.5 times the current atmospheric CO_2 level to 585 $\mu L \cdot L^{-1}$. Photosynthetic light curves were carried out at a temperature of 35˚C, 40°C and 45°C. The final CO_2 manipulation raised the leaf chamber CO_2 level to twice the current atmospheric level to 780 $\mu L \cdot L^{-1}$. Photosynthetic light curves were again measured for the three temperature conditions using the same procedure as above. Finally, CO_2 response curves were measured at a light level approximating canopy shade (700 $\mu mol \cdot m^{-2} \cdot s^{-1}$). This was first completed at 35°C and then at 40°C and 45°C as described for the light curves.

Both Microsoft Excel© and JMP© IN 5.1 were used for data organization and analysis. The JMP© IN 5.1 software was used to determine significant differences using a repeated measures MANOVA on the curves of the photosynthetic rate, intercellular CO_2 concentration, stomatal conductance and transpiration using the light level, PAR, as the repeat variable (Sall et al. 2011). Water use efficiency was calculated by dividing the photosynthetic rate by the transpiration rate and also analyzed using a repeated measures MANOVA. Significance levels for all tests were $P \leq 0.05$. All data were checked for normality with the Shapiro-Wilk W test and homogeneity of variance with Bartlett's test and when necessary log transformed. A standard least squared ANOVA was used to determine significant differences in each light response curve at each different CO_2 concentration and temperature combination examined. All ANOVAs for light response curves were significant ($P \leq 0.05$).

Additional characteristics were determined including the maximum photosynthetic rate (A_{max}) which was the highest A_{net} measured for each replicate or a mean of the highest A_{net} values that were not significantly different. The dark respiration rate (R_d) was the gas exchange rate at PAR = 0 $\mu mol \cdot m^{-2}\, s^{-1}$. The initial slope (*IS*) or initial response, also known as the quantum yield (Φ) was the linear relationship calculated using the dark values and A_{net} at increasing PAR until the regression coefficient of the slope decreased. The light compensation point (L_{cp}) was calculated as the PAR when A_{net}= 0 μmol $CO_2 \cdot m^{-2}\, s^{-1}$ using the linear regression of the initial response. The light saturation point (L_{sat}) was the light level when the initial slope reached A_{max}. A standard least squared ANOVA was used to determine significant

differences for the CO_2 concentration and temperature effects. Tukey-Kramer HSD multiple comparison tests were used to determine differences between pair wise comparisons (Sall et al. 2011).

RESULTS

All canopy trees were identified and counted and the communities were simple with two major overstory species. Relative canopy density of *Juniperus ashei* was 61 ± 12% (mean ± standard deviation). Relative canopy density of *Quercus virginiana* was 36 ± 6%. Other canopy species found in one or two stands were *Diospyros texana* (Texas persimmon), *Celtis laevigata* (sugarberry or hackberry), *Ulmus crasifolia* (cedar elm), *Prosopis glandulosa* (mesquite) and *Sophora secundiflora* (Texas mountain laurel) with relative densities of 0.4 – 1.8%. *Garrya ovata* was not present in the canopy, it was present in the understory of 10/11 stands examined with a relative density of 0.12 ± 0.04% and density of 120 plants/ha.

Light curve results of the main effects of CO_2 concentration and temperature were compared. Response variables including photosynthetic rate, stomatal conductance, dark respiration rate, intercellular CO_2 concentration and water use efficiency were significant only for CO_2 concentration and not temperature. Interactions were not significant and removed from the model (Table 1).

Main effects	Photo.Rate	*Conduction*	*Resp.*	[CO_2]	Transpir.	WUE
Temperature	0.9425	*0.5153*	*0.3346*	0.5427	0.2331	0 .2143
[CO_2]	**<0.0001**	**0.0038**	**0.0314**	**<0.0001**	0.1376	**0.0014**

Table 1. Table includes *P*-values for repeated measures MANOVAs of gas exchange measurements for *Garrya ovata* comparing the main effects of temperature and CO_2 at 16 light levels (interactions were not significant and removed from the models). Data is from three replicates at three concentrations of CO_2 (390, 585 and 780 $\mu L \cdot L^{-1}$) and three temperatures (35, 40 and 45 °C). Bold entries are significant.

The mean curves of the photosynthetic rates are shown by temperature and CO_2 effects (Figure 1A and 1B). Photosynthetic rates compared by temperature were not significantly different (MANOVA, $P = 0.9364$) (Figure 1A). However, photosynthetic rates increased to a plateau of approximately 9 μmol $CO_2 \cdot m^{-2} s^{-1}$ as light levels increased. The comparisons by CO_2 concentration were statistical different between the curves (repeated measures MANOVA, $P = 0.0010$) (Figure1B). The curves increased as the light levels increased and as the CO_2 concentration increased. The ambient (390 $\mu L \cdot L^{-1}$) or low CO_2 concentration was significantly different from both the middle (585 $\mu L \cdot L^{-1}$) and the high (780 $\mu L \cdot L^{-1}$) CO_2 concentration ($P = 0.0033$ and $P = 0.0009$). The middle (585 $\mu L \cdot L^{-1}$) and the high (780 $\mu L \cdot L^{-1}$) CO_2 concentration were not significantly different from each other ($P = 0.4582$). Between the ambient CO_2 concentration (390 $\mu L \cdot L^{-1}$) and the middle CO_2 concentration the highest photosynthetic rate increased approximately 35% while between the middle and the high CO_2 concentration the rate increased 11% for a total increase of 46% (Figure1B).

Transpiration rate was not affected by increasing temperature or CO_2 level as light levels were increased ($P = 0.2331$ and 0.1376 respectively). Stomatal conductance was not affected by increasing temperatures as light levels changed ($P = 0.5153$). Stomatal conductance did differ significantly by CO_2 level but differences were small and are not presented ($P = 0.0038$).

The mean curves of the calculated water use efficiencies are displayed by temperature and CO_2 concentration (Figure 2A and 2B). Water use efficiency did not differ significantly by temperature ($P = 0.2143$) (Figure 2A), but did differ significantly by CO_2 level ($P = 0.0014$) (Figure 2B). As light levels and the CO_2 concentration increased the water use efficiency increased. The water use efficiency at ambient CO_2 did not show a significant increase compared to the middle CO_2 concentration (17%) but did

increase significantly compared to the high CO_2 concentration for a total increase of approximately 37% (Figure 2B).

Measured light curve parameters including photosynthetic maximum (A_{max}), light saturation point (L_{sp}), light compensation point (L_{cp}), dark respiration (R_d) and initial slope (IS) were compared with the standard least squared ANOVA (Table 2). Temperature and CO_2 concentration were main effects. For each of the comparisons the interactions were not significant and removed from the model. None of the temperature comparisons were significant (Table 2). The A_{max}, L_{sp} and R_d were found to be significant by CO_2 concentration with no other CO_2 comparisons being significant (Table 2).

Main effects	A_{max}	L_{sp}	L_{cp}	R_d	IS
Temperature	0.9425	0.5734	0.6895	0.3346	0.8541
CO_2 concentration	**<0.0001**	**<0.0001**	0.0897	**0.0314**	0.9268

Table 2: Table of *P*-values for Standard Least Squared ANOVAs for measured light curve parameters for *Garrya ovata* including the main effects of temperature and CO_2 (interactions were not significant and removed from the models). Data is from three replicates at three concentrations of CO_2 (390, 585 and 780 $\mu L \cdot L^{-1}$) and three temperatures (35, 40 and 45 °C). Bold entries are significant.

The maximum photosynthetic rate (A_{max}) did not change with temperature ($P = 0.9425$) while it did increase significantly with CO_2 concentration ($P < 0.0001$) (Table 2; Figure 3). Temperature had little effect on the mean A_{max} with values of 9.94, 10.15 and 10.25 ± 0.64 μmol CO_2 $\cdot m^{-2}$ s^{-1}. Tukey comparisons of the CO_2 effect showed significant differences between the ambient CO_2 concentration and both the middle and high CO_2 concentrations (Figure 3). The A_{max} increased from 7.06 $\mu molCO_2 m^{-2} s^{-1}$ for the ambient CO_2 concentration to 10.90 μmol CO_2 m^{-2} s^{-1} for the middle CO_2 concentration and again to 12.38 $\mu molCO_2$ m^{-2} s^{-1} for the high CO_2 concentration. This was an A_{max} increase of approximately 35% from the low to medium CO_2 concentration and another 12% increase from medium to high CO_2 concentration giving an overall increase of approximately 47%. The interaction term was not significant ($P = 0.9975$). The interaction plot of A_{max} did not show a trend for temperature at any CO_2 concentration (Figure 3).

The light saturation point (L_{sp}) did not change significantly with temperature ($P = 0.5734$) (Table 2; Figure 4). The mean L_{sp} for the three temperatures were 375.0, 341.7 and 348.4 ± 23.4 $\mu mol \cdot m^{-2}$ s^{-1} (Figure 4). [CO_2] had a significant effect on L_{sp} ($P< 0.0001$) (Figure 4). A significant difference was found between values for the ambient CO_2 L_{sp} (245.6 $\mu mol \cdot m^{-2} s^{-1}$) and both the medium CO_2 L_{sp} (374.3 $\mu mol \cdot m^{-2}$ s^{-1}) and high CO_2 L_{sp} (445.1 $\mu mol \cdot m^{-2}$ s^{-1}). The Tukey comparisons showed no significant differences for L_{sp} between the middle and the high CO_2 concentrations (Figure 4). The interaction term was not significant ($P = 0.7757$). The interaction graph for L_{sp} did not show a trend for temperature at any CO_2 concentration (Figure 4). Light saturation increased approximately 59% from ambient to high CO_2.

There were no significant difference for the light compensation point (L_{cp}) values (25.4, 25.5 and 29.0 ± 3.4 $\mu mol \cdot m^{-2} s^{-1}$) as the temperature increased (35, 40 and 45 °C respectively) ($P = 0.6895$). The light compensation point increased slightly as the CO_2 concentration increased (21.3, 26.3 and 32.3 $\mu mol \cdot m^{-2}$ s^{-1}) but this trend was marginal ($P = 0.0897$). The interaction term was not significant ($P = 0.9460$), thus the interaction graph for L_{cp} did not show a trend for temperature at any CO_2 concentration.

For the dark respiration rate (R_d) there was a non-significant trend of increasing values (0.72, 0.78 and 0.89 ± 0.079 μmol CO_2 $\cdot m^{-2}$ s^{-1}) as the temperature increased from 35 to 40 and 45 °C respectively ($P = 0.3346$) (Figure 5). The trend for CO_2 concentration was significant. The R_d at the ambient CO_2 concentration (0.63 μmol CO_2 $\cdot m^{-2}$ s^{-1}) was significantly different from the R_d at the high CO_2

concentration (0.95 µmol CO_2 $\cdot m^{-2}$ s^{-1}) while the medium CO_2 R_d (0.80 µmol CO_2 $\cdot m^{-2}$ s^{-1}) was not significantly different from either the ambient or the high CO_2 R_d (Figure 5). The interaction term was not significant ($P = 0.9856$) and when removed from the model results did not change. The R_d increased approximately 34% from ambient to high CO_2.

The *IS* did not differ significantly with temperature or CO_2 concentration and results are not presented. There were no statistical difference as temperature changed but IS values were 0.031, 0.034 and 0.033 µmol CO_2 $\cdot m^{-2}$ s^{-1}/µmol$\cdot m^{-2}s^{-1}$ ($P = 0.8541$). CO_2 concentration did not have a significant effect on the *IS* and values were 0.033, 0.033 and 0.031 µmol CO_2 m^{-2} s^{-1}/µmol m^{-2} s^{-1} ($P = 0.9268$). The interaction term was not significant ($P = 0.9835$) and removed from the model and the results did not change.

When light levels were held constant at sub canopy levels (700 µmol$\cdot m^{-2}\cdot s^{-1}$) gas exchange rates increased with CO_2 concentrations but the response to elevated temperatures were not significant and differences were small (Figure 6). CO_2 uptake increased as the CO_2 concentration increased but there was no significant difference ($P > 0.05$) between temperature treatments (35, 40 and 45 °C). The highest CO_2 concentrations tested (1000 and 1200 µL$\cdot L^{-1}$) had higher amounts of variation around the mean then the lower levels. Once the CO_2 concentration reached 585 - 700 µL$\cdot L^{-1}$, the photosynthetic rates reached a plateau of approximately10 µmol CO_2 $\cdot m^{-2}$ s^{-1}.

None of the repeated measures MANOVAs performed on the photosynthetic response, intercellular CO_2 concentration, stomatal conductance and transpiration were significantly different by temperature ($P > 0.05$ for all). Both CO_2 uptake and intercellular CO_2 concentration increase with increasing CO_2 concentration but not to changing temperature. The stomatal conductance and transpiration curves mimicked each other seemingly following the same overall trend (not presented).

Temperature had little effect on the overall CO_2 photosynthetic response curves ($P = 0.8672$). The curves exhibit a fairly linear relationship from the low CO_2 concentration (50 µL$\cdot L^{-1}$) to approximately 600 µL$\cdot L^{-1}$. Once the CO_2 concentration reached 585 - 700 µL$\cdot L^{-1}$the photosynthetic rates reached a plateau of approximately 10 µmol CO_2 m^{-2} s^{-1}.

The repeated measures MANOVA of the water use efficiency response to temperature was not significant ($P = 0.4885$). The curves increased from a low of approximately -0.15 mmol H_2O·mol CO_2^{-1} at 50 µL$\cdot L^{-1}$to a plateau of approximately 3.2 mmol H_2O·mol CO_2^{-1} at 585 µL$\cdot L^{-1}$which was maintained until a peak value of 4.2 mmol H_2O·mol CO_2^{-1} was reached at the 1000 µL$\cdot L^{-1}$of CO_2 (Figure 7).

DISCUSSION

Based on our results and comparisons with literature values with other species (Hull 2002; Wayne and Van Auken 2012), *Garrya ovata* appears to be a sun adapted species (Valladares and Niinemets 2008; Van Auken and Bush 2009). Our experiments showed increased CO_2 uptake at higher light levels (Figure 1). In addition, increasing atmospheric CO_2 caused increased plant CO_2 uptake especially at higher light levels (Figure 1) also suggesting that *G. ovata* is a sun plant. This does not explain the low density of *G. ovata* found below the canopy of these woodland communities or the lack of *G. ovata* in the grassland gaps and patches. Density of large native herbivores have increased dramatically in central Texas over the past few decades as well as in many other areas of North America causing community structural and composition changes (Anderson and Loucks 1979; Doughty 1983; Augustine and Frelich 1998; Russel et al. 2001; Cadenasso et al. 2002; Abrams 2003; Krueger et al. 2009; Kain et al. 2011; Abrams and Johnson 2012). Plant species in the study areas have been shown to be susceptible to herbivory and require exclosures to maintain populations (Van Auken 1988, 1993; Leonard and Van Auken 2013). This is similar to what is happening to *Quercus* sp. and a number of other woody species in

many North American forests (Anderson and Loucks 1979; Abrams 2003; Abrams and Johnson 2012). Although herbivory of *G. ovata* was not directly examined in this study there is a high potential for herbivory (browsing) to have a major effect on the population structure and distribution of this species.

The light level responses found in this study suggest that *G. ovata* is similar to other geographically restricted eastern North American low density species reported to be shade intolerant and susceptible to herbivory (Baskin and Baskin 1988). The response of *G. ovata* to canopy position (open grassland or below a canopy) is probably dependent on the presence of herbivores and *G. ovata's* current low relative density is caused by a high density of large native herbivores (probably white tailed deer). Presence of some *G. ovata* plants below the canopy maybe because of the presence of the high levels of other woody shrubs below the canopy that could afford some level of protection from the herbivores, masking their presence and position similar to what has been reported for other species (Leonard and Van Auken 2013).

These central Texas *Juniperus* woodlands seem to be successional communities (Van Auken 2009). In the eastern North American deciduous forests *Juniperus* plants are often found in forest gaps or blow downs or on shallow soil in glades (Van Auken and McKinley 2008). In western North America *Juniperus* tend to occur above the desert communities and above the arid or semiarid grasslands, but usually below the higher elevation pine, spruce or fir forests (Van Auken 2000b). In central Texas *Juniperus ashei* establishes on hillsides and in former grasslands usually on shallow soil (Terletzky and Van Auken 1996). The presence of *Juniperus* woodlands in central Texas and many other parts of the world today has probably been caused by a number of factors with constant high levels of domestic grass herbivory and a concomitant reduction of grassland fire frequency being paramount (Van Auken 2000B, 2009).

Grass plants and grasslands are favored when grassland biomass and fire frequency are relatively high while woody plants, like various species of *Juniperus,* and many species of nitrogen fixing legumes, like *Prosopis*, *Senegalia* and *Vachellia* (formerly *Acacia*), are favored when fire frequency is low or nonexistent (Bond 2008; Van Auken and Bush 2013). Apparently the composition of many grasslands and savannas has changed and the direction of community succession has changed because of a new set of conditions controlling the structure and composition of these communities (Begon et al. 2006; Bond 2008; Smith and Smith 2012; Van Auken and Bush 2013).

These new, recent conditions in the grasslands which include high and constant levels of herbivory by domestic animals resulting in low levels of light, fluffy fuel and a concomitant reduction in fire frequency have resulted in woody plant establishment. In the woodlands, browsing by native herbivores is causing community change. These conditions will be further modified in the future by higher levels of atmospheric CO_2 and higher temperatures (NCA 2014). These new conditions will allow some species to expand their aerial cover and increase their density, while other species will decline. The result will be changes in community composition and structure. A species in these central Texas *Juniperus/Quercus* communities expected to change in density and basal area is *G. ovata*. It has a high gas exchange rate which we have shown to increase with increased light levels and increased atmospheric CO_2 levels. But, there seems to be a factor or factors that we have not examined or accounted for which is natural herbivory or browsing by white-tailed deer (*Odocoileus virginianus*). This species has been shown to cause establishment difficulties for herbaceous species in many areas of central Texas (Leonard and VanAuken 2013).This difficulty in establishment has also been shown for many woody species possibly in conjunction with competition for water with C_4 southern grasses (Van Auken 1993; Russel et al. 2001; Cadenasso et al. 2002; Krueger et al. 2009; Kain et al. 2011). However, predicting future plant community changes and rates of species change will be an arduous task.

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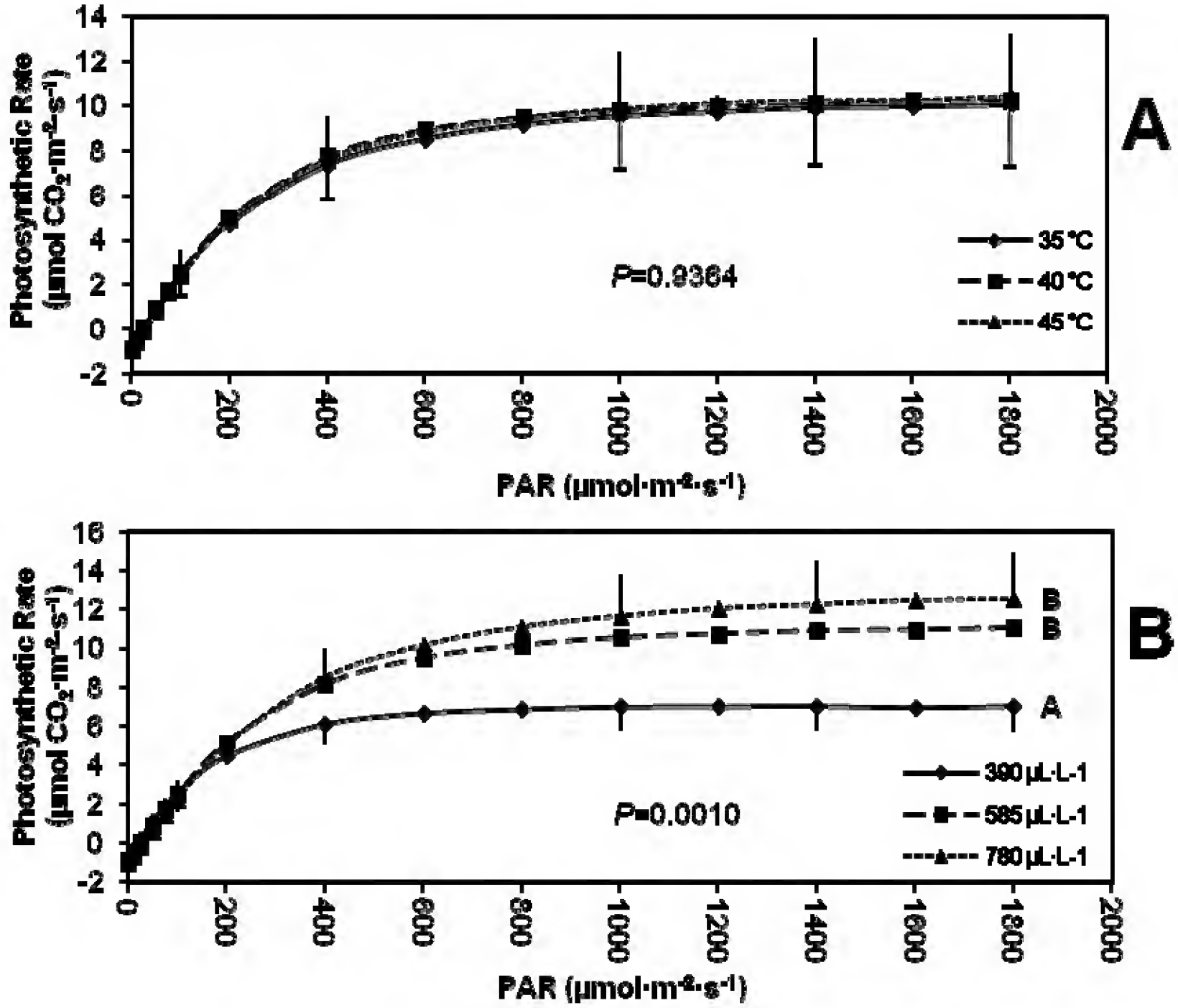


Figure 1: Mean repeated measures MANOVA curves of the photosynthetic rates for *Garrya ovata* displayed by temperature (A) and CO_2 concentration (B) treatment for main effects only. *P*-values are shown from the repeated measures MANOVA for the main effects (Temperature and CO_2). Like letters at the end of the curves indicate no significant difference. Data is from three replicates at three concentrations of CO_2 (390, 585 and 780 $\mu L \cdot L^{-1}$) and three temperatures (35, 40 and 45 °C). Representative error bars are shown indicating standard deviations.

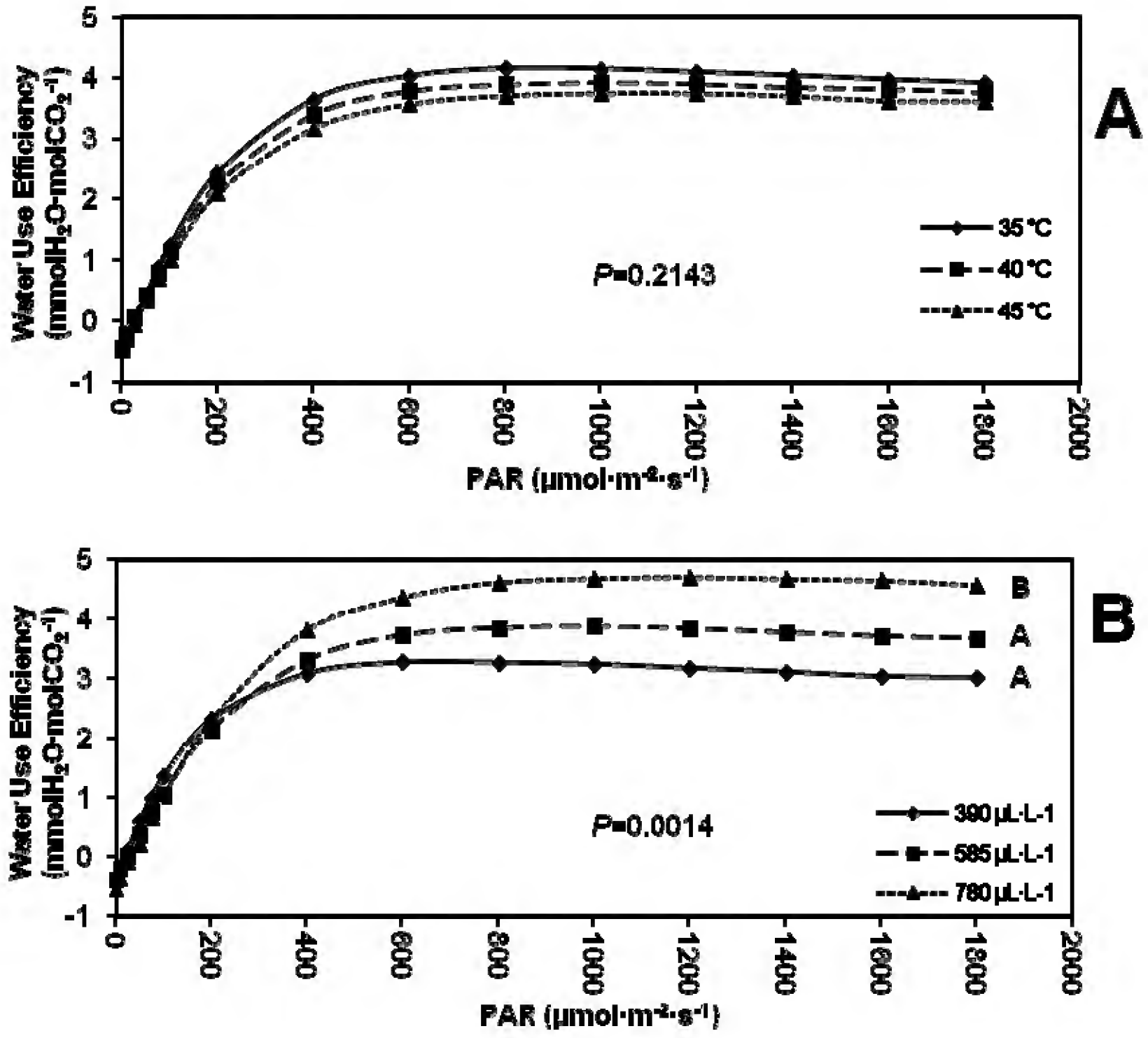
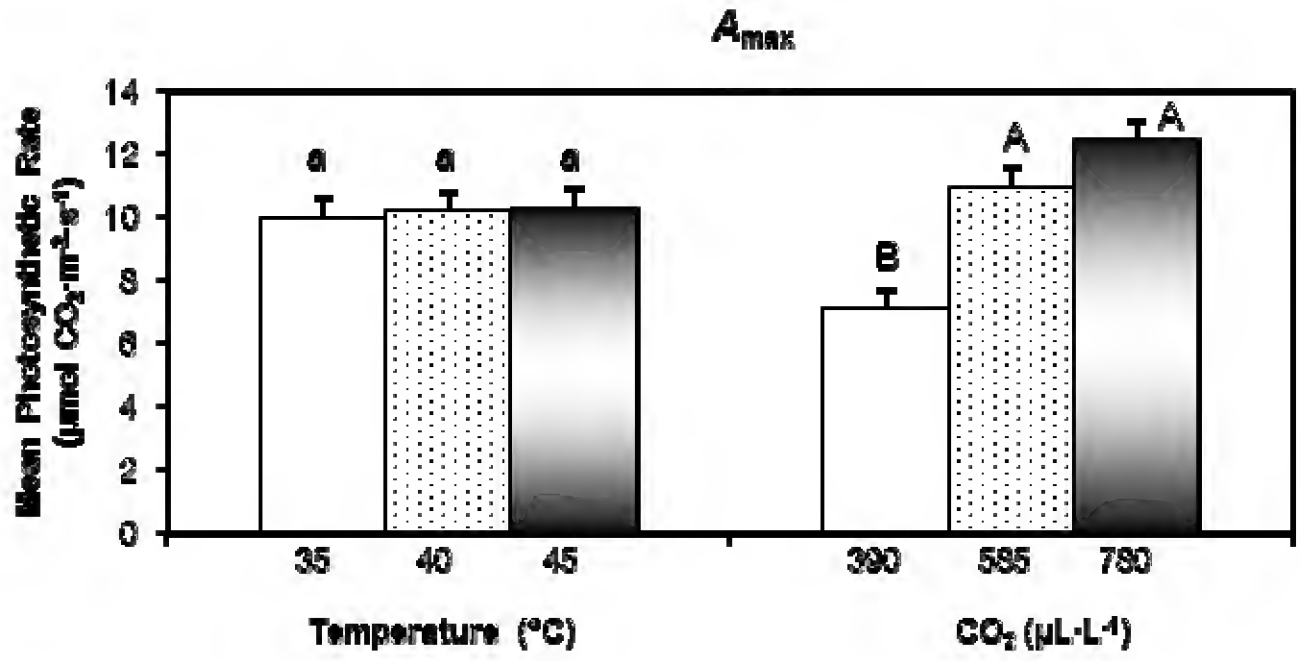


Figure 2: Mean repeated measures MANOVA curves of the water use efficiency for *Garrya ovata* displayed by temperature (A) and CO_2 (B) treatment for main effects only. *P*-values are shown from the repeated measures MANOVA for the main effects (Temperature and CO_2). Data is from three replicates at three concentrations of CO_2 (390, 585 and 780 $\mu L \cdot L^{-1}$) and three temperatures (35, 40 and 45 °C).



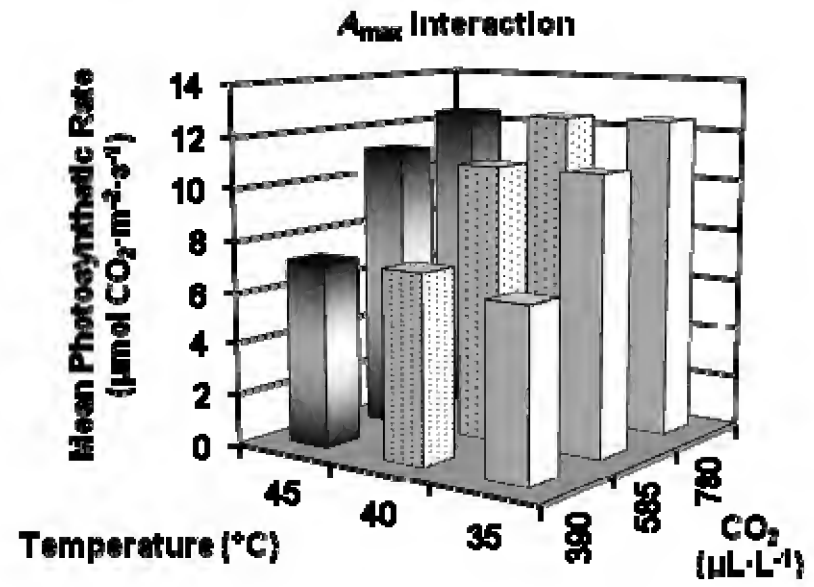
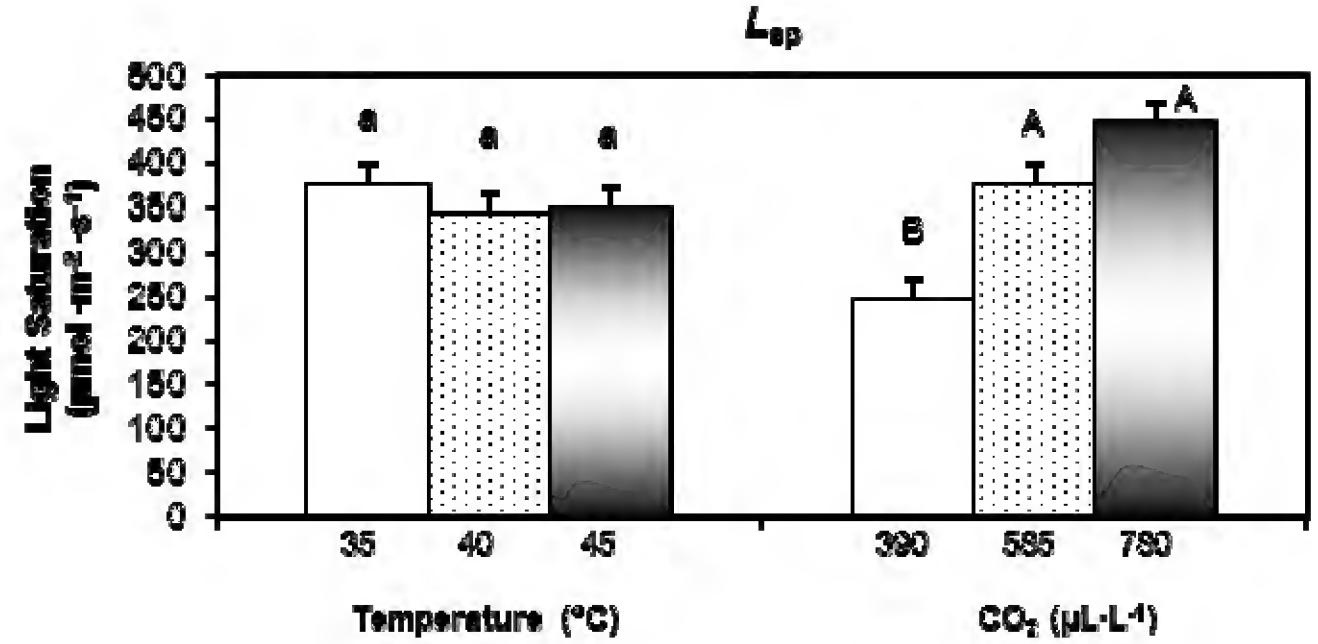


Figure 3: Standard least squared ANOVA results for the *Garrya ovata* A_{max} comparisons. Temperature and CO_2 graphs are for main effects only with the Tukey comparisons labeled. Temperature and the interaction are not significant ($P = 0.9425$ and $P= 0.9975$) while the CO_2 concentration is significant ($P< 0.0001$). Like letters from the Tukey comparisons indicate no significant difference between the means. Data is from three replicates at three levels of CO_2 (390, 585 and 780 $\mu L \cdot L^{-1}$) and three temperatures (35, 40 and 45 °C).



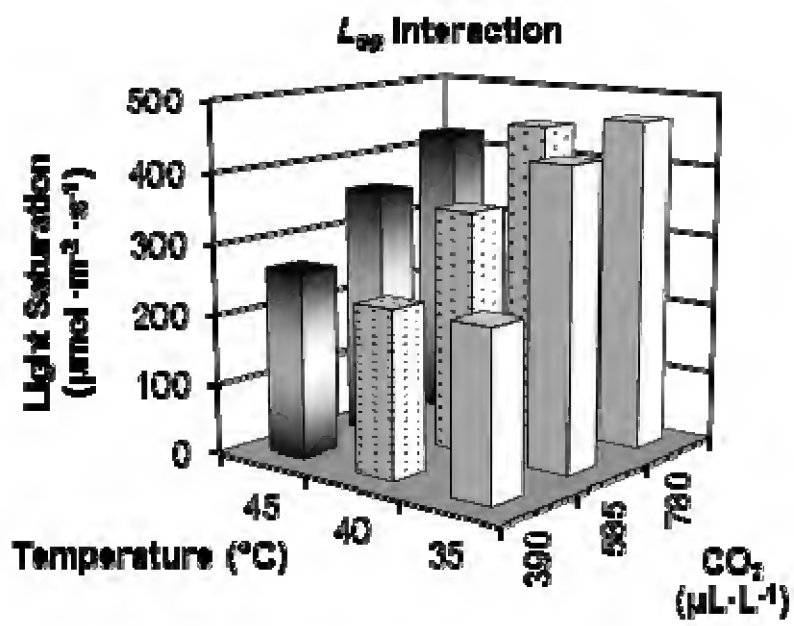


Figure 4: Standard least squared ANOVA results for the *Garrya ovata* L_{sp} comparisons. Temperature and CO_2 graphs are for main effects only with the Tukey comparisons labeled. Temperature and the interaction are not significant ($P = 0.5734$ and $P = 0.7757$) while the CO_2 concentration is significant ($P < 0.0001$). Like letters from the Tukey comparisons indicate no significant difference between the means. Data is from three replicates at three concentrations of CO_2 (390, 585 and 780 $\mu L \cdot L^{-1}$) and three temperatures (35, 40 and 45 °C).

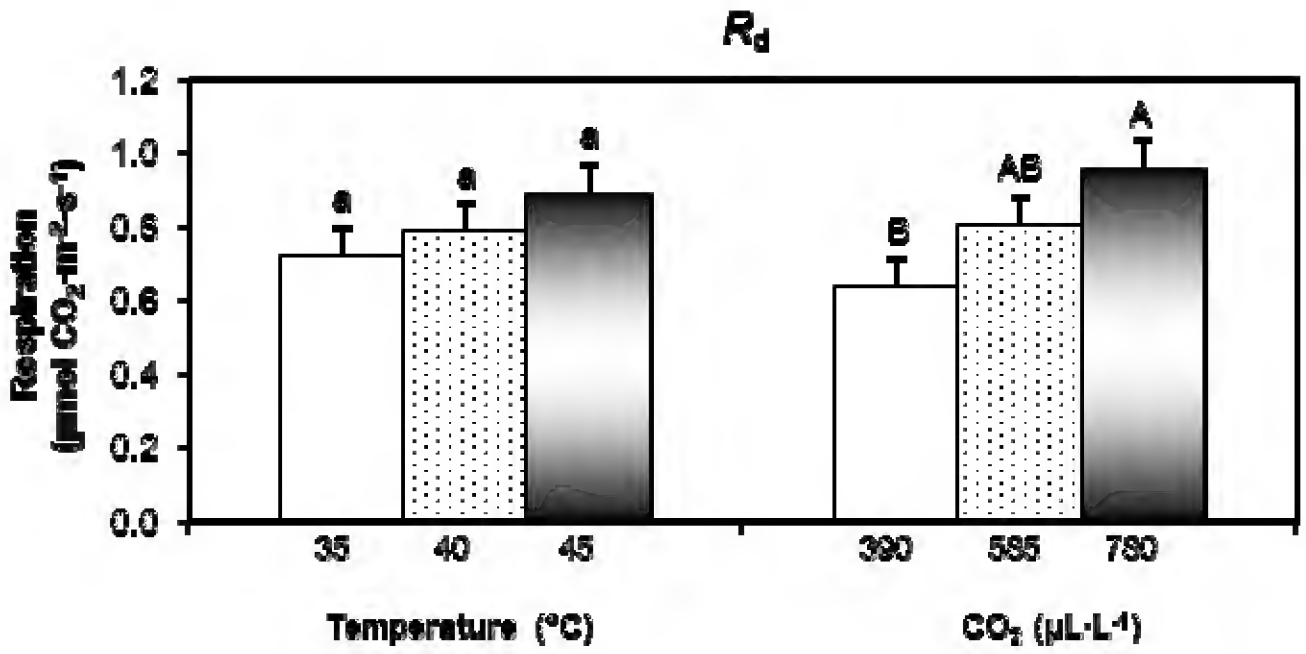


Figure 5: Standard least squared ANOVA results for the *Garrya ovata* R_d comparisons. Temperature and CO_2 graphs are for main effects only with the Tukey comparisons labeled. Temperature and the interaction were not significant ($P = 0.3346$ and $P = 0.9856$). The CO_2 concentrations were significantly different ($P = 0.0314$). Like letters from the Tukey comparisons indicate no significant difference between the means. Data is from three replicates at three concentrations of CO_2 (390, 585 and 780 $\mu L \cdot L^{-1}$) and three temperatures (35, 40 and 45 °C).

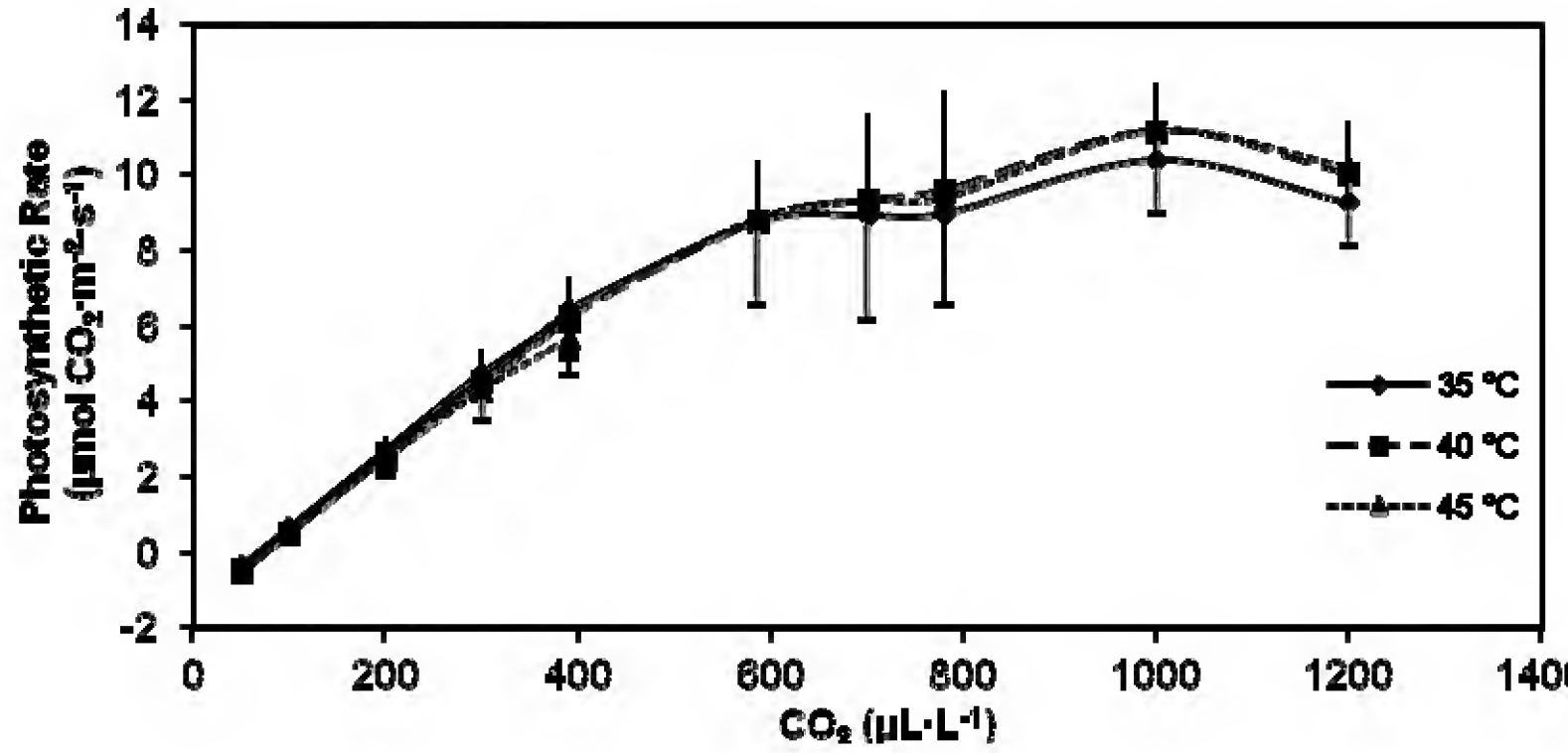


Figure 6: CO_2 response curves for *Garrya ovata* at a light level of 700 μmol·m^{-2}·s^{-1} and three temperatures (35, 40 and 45 °C). The repeated measures MANOVA of the calculated CO_2 response was not significant by temperature (P = 0.8854). A standard least squared ANOVA was used to determine significant differences in each light response curve for each temperature. All three ANOVAs were significant ($P \leq$ 0.05). Each curve was plotted from a mean of three replicates. Error bars are shown indicating standard deviation with the open end (|) for the upper most curve and the bar end (┬) for the lower curve.

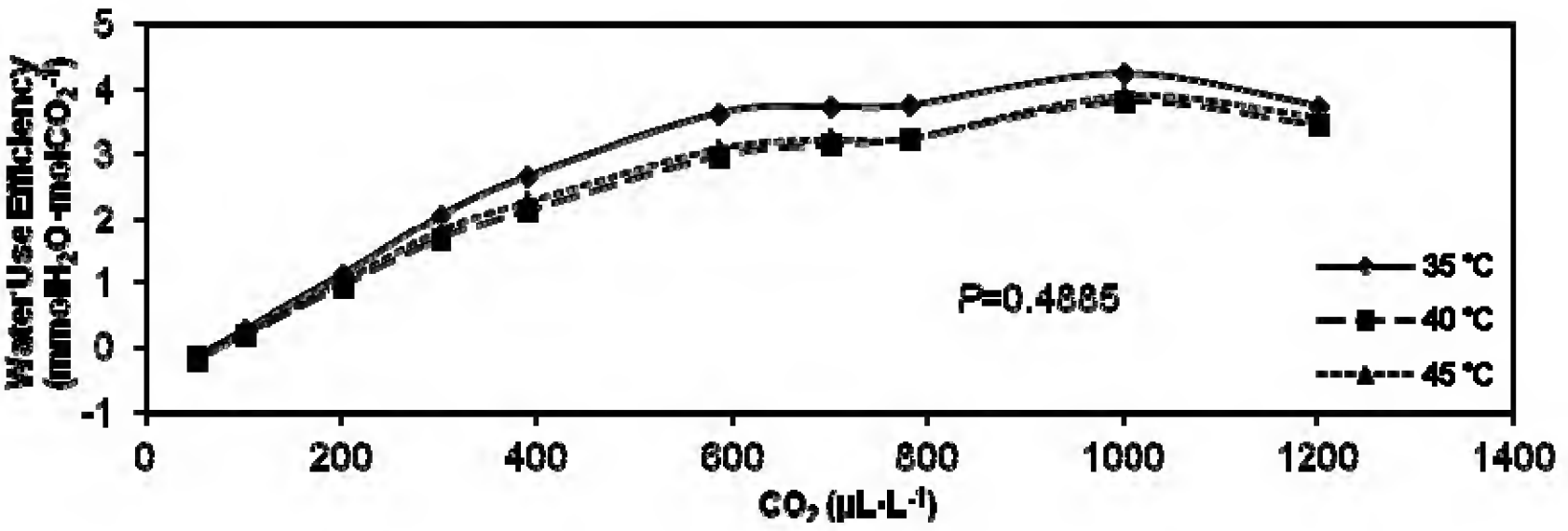


Figure 7: Repeated measures CO_2 response curves of the water use efficiency for three replicates of *Garrya ovata* at a light level of 700 μmol·m^{-2}·s^{-1} and three temperatures (35, 40 and 45 °C). Temperature was not significant ((P = 0.4885). A standard least squared ANOVA was used to determine significant differences in each light response curve for each temperature. All three ANOVAs were significant ($P \leq$ 0.05). Each curve was plotted from a mean of three replicates. Error bars are shown indicating standard deviation with the open end (|) for the upper most curve and the bar end (┬) for the lower curve.

New Names for the Texas Taxa of Acacia (Fabaceae)

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712

ABSTRACT

Recent DNA studies of numerous authors (Arce & Bachman 2006; Miller and Bayer, 2003; Seigler, Ebinger and Miller 2006; Seigler Ebinger and Glass, 2012; etc.) have realigned the classically conceived **Acacia**, recognizing from its midst several genera. Amongst the Texas taxa numerous name changes are in order, these having been proposed by various authors, all of these treated as **Acacia** in my Legumes of Texas (Turner, 1959), and in my Atlas of the Vascular Plants of Texas (Turner et al. 2003). Distribution maps of the **Senegalia greggii** complex in Texas are also provided. *Phytologia 97(2): 120-122 (April 1, 2015).* ISSN 030319430.

KEY WORDS: *Acacia, Acaciella, Senegalia, Vachellia*

The following name changes of Texas **Acacia**, sensu Turner (1959; 2003) have been proposed by various authors, largely on the basis of DNA data.

Acacia angustissima (Mill.) Kuntze

Acaciella angustissima (Mill.) Britton & Rose

var. **chisosiana** (Isely) B.L. Turner, **comb. nov.**
based upon *Acacia angustissima* var. *chisosiana* Isely, Sida 3: 370. 1969.

var. **hirta** (Nutt.) B.L. Turner, **comb. nov.**
based upon *Acacia hirta* Nutt., in Torr. & Gray, Fl. N. Amer. 1: 404. 1840.
[includes *Acacia texensis* Torr. & Gray; cf. Turner 1996]

var. **filicoides** (Cav.) L. Rico, Kew. Bull. 59: 327. 2004.

Acacia berlandieri Benth.

Senegalia berlandieri (Benth.) Britton & Rose

Acacia greggii A. Gray

Senegalia greggii (A. Gray) Britton & Rose

Acacia roemeriana Scheele

Senegalia roemeriana (Scheele) Britton & Rose

Acacia wrightii Benth.

Senegalia wrightii (Benth.) Britton & Rose

Seigler, Ebinger and Miller (2006) treated this taxon at the specific level; Turner (1996) inexplicably treated the taxon at the varietal level (as *A. greggii* var. *wrightii*) and mapped it as such in his Atlas of Texas Plants (2003). Seigler, Ebinger and Glass (2012) are clearly correct in their interpretation of the complex; my restudy of the distribution of two taxa in Texas, based upon their criteria, is shown in Figs. 1 and 2.

Acacia constricta Benth. ex A. Gray

Vachellia constricta (Benth.) Seigler & Ebinger

Acacia farnesiana (L.) Willd.

Vachellia farnesiana (L.) Wight & Arn.

Acacia rigidula Benth.

Vachellia rigidula (Benth.) Seigler & Ebinger

Acacia schaffneri S. Wats.

Vachellia bravoensis (Isley) Seigler & Ebinger

Acacia schottii Torr.

Vachellia schottii (Torr.) Seigler & Ebinger

Acacia vernicosa Britton & Rose.

Vachellia vernicosa (Britton & Rose) Seigler & Ebinger

Hybrid taxa

Acacia emoryana Benth.

Senegalia x emoryana (Benth.) Britton & Rose
[reportedly hybrid of **S. berlandieri x S. greggii**]

Senegalia x turneri Seigler, Ebinger & Glass
[reportedly hybrid of **S. berlandieri x S. wrightii**]

I have accepted the collections (cited in Seigler, Eblinger and Glass 2012) of the above hybrids; these are mapped accordingly in my revised Atlas.

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Seigler, D.S., J.E. Ebinger and C. Glass. 2012. *Senegalia, berlandieri, S. greggii,* and *S. wrightii* hybrids (Fabaceae: Mimosoideae) in Texas and adjacent Mexico. Phytologia 94: 439-455

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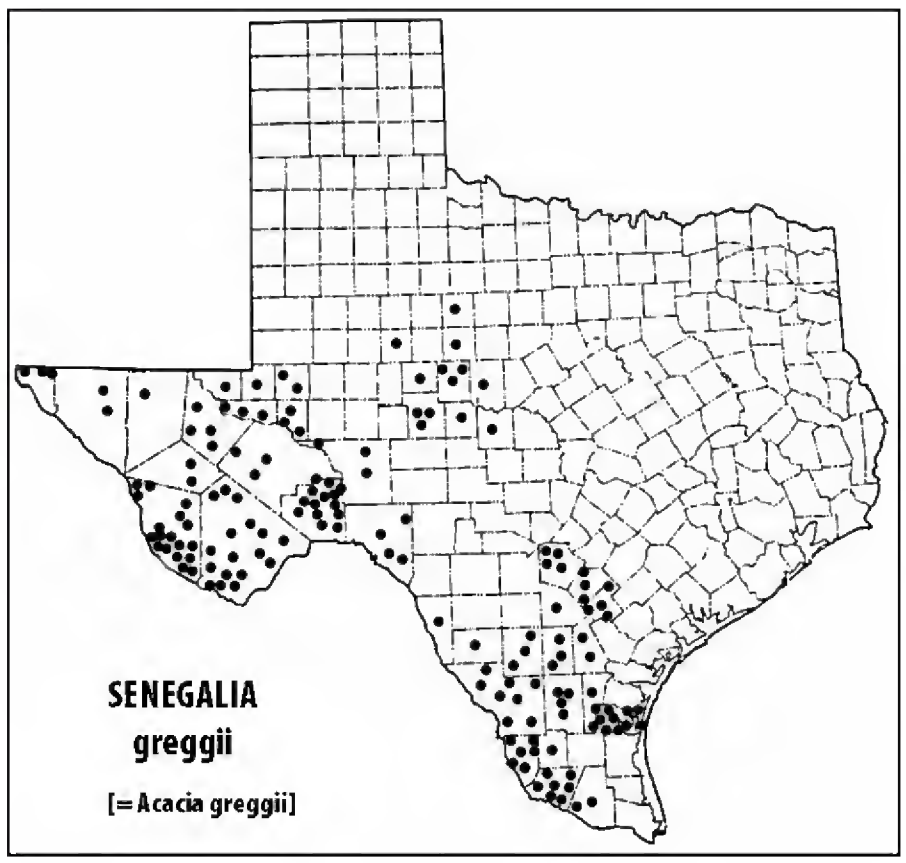

Fig.1. Distribution of *Senegalia greggii.*

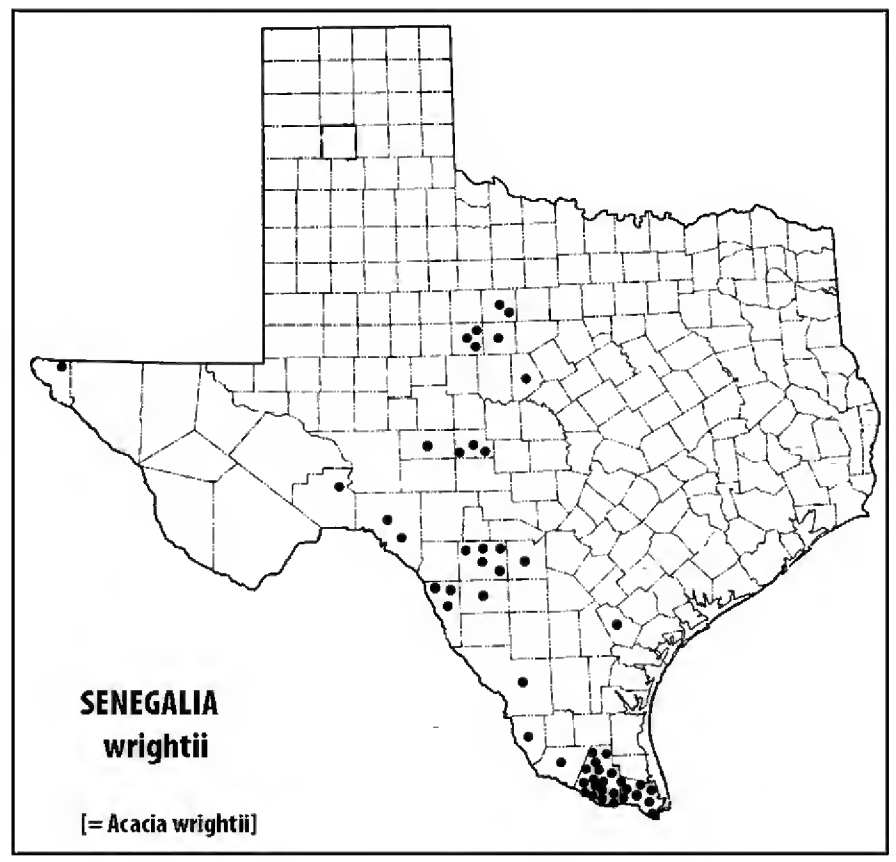

Fig. 2. Distribution of *Senegalia wrightii.*

Juniperus communis in Morocco: analyses of nrDNA and cpDNA regions

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
Robert_Adams@baylor.edu

Mustapha Rhanem
Moulay Ismail University, Faculty of Science, PB 11201 Zitoune, Meknes, 50000, Morocco

and

Andrea E. Schwarzbach
Department of Biomedicine, University of Texas at Brownsville, Brownsville, TX 78520, USA.

ABSTRACT

Juniperus communis from Morocco was analyzed by DNA sequence data from nrDNA plus four cp DNA regions (4315 bp) and found to be in a clade with other *J. communis* from Europe. The sub-alpine, prostrate *J. communis* plants of Morocco, each only contained a single SNP and a one bp indel (deletion) in the trnSG sequence data for a total of 2 MEs (mutation events). The Moroccan prostrate *J. communis* appear to be a variant of *J. communis* of Europe and central Asia and not part of the var. *hemispherica* group. Published on-line **www.phytologia.org** *Phytologia 97(2): 123-128 (April 1, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus communis* forma *pygmaea, J. communis, J. oblonga, J. pygmaea,* Azerbaijan, nrDNA, cpDNA sequences, taxonomy.

Juniperus communis is a circumboreal species with perhaps the largest distribution of any conifer. Its habit ranges from upright trees (in Europe) to shrubs, to prostrate shrubs (Adams, 2014). In spit of the variation in habit, few differences in its DNA have been found (see Adams, 2014 for discussion).

Adams and Tashev (2013) compared the leaf essential oils of *J. communis, J. pygmaea* and *J. sibirica* from Bulgaria with the oils of *J. communis* of Sweden and *J. saxatilis* of Switzerland. From their analysis, the oils do not ordinate *J. communis, J. pygmaea* and *J. sibirica* from Bulgaria into separate groups, but they are generally interspersed. Additional research (Adams, Tashev and Schwarzbach, 2014) using DNA sequences from nrDNA and four cp regions gave no clear separation of *'pygmaea'* from *J. communis* and *J. c.* var. *saxatilis*. They concluded that the shrubby habit is likely controlled by only a few genes and recognized the taxon as *J. communis* f. *pygmaea* (K. Koch) R. P. Adams and A. N. Tashev.

Recently, Adams et al. (2015) compared the DNA sequences of putative *J. communis 'pygmaea'* from Azerbaijan and found it in a clade with *J. communis 'oblonga'* from Armenia, not with *J. communis* forma pygmaea of Bulgaria. They elected to not recognize this variant, but to treat it as *J. communis*.

While doing routine field work one of the authors (MR) found and collected samples from unusual, prostrate *J. communis* plants growing at 3000 m in the Atlas Mtns. of Morocco (Fig. 1). Examination of the specimens and photos by senior author (RPA), raised the question if these might be part of var. *hemispherica* as found in Mt. Etna, Sicily and the Sierra Nevada, Spain.

Figure 1. Habitat of *J. communis* in the high Atlas Mtns., Morocco (3000m). Notice the plants are very prostrate. This may be due to winter temperatures.

The purpose of this study was to compare data from nrDNA and four cpDNA regions of *J. communis* from Morocco with other members of *Juniperus* sect. *Juniperus* from the eastern hemisphere to determine the taxonomic affinity of *J. communis* from Morocco.

MATERIALS AND METHODS

Plant material - Morocco: *J. communis, Adams Lab acc 14222-14226, (M. Rhanem ns),* common on exposed slopes, prostrate plants, High Atlas Mtns., 32° 31.5' N; 4° 57' W. elev. 3000m, Morocco. Bulgaria, *J. communis* var. *communis*, *Adams Lab acc 13730-31, 14058-60, (Alex Tashev, 2012-JC1-5)*, Eastern Rhodopes, in protected site "Gumurdjinsky Shezhnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca,* 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m. *J. communis* f. *pygmaea*, *Adams Lab acc. 13734-35, 14064-66,(Alex Tashev, 2012-JP1-5)*, Central Rhodopes. Mursalitza part, locality "Piramidata". On high-mountain meadow, on a limestone rock near a forest of *Pinus sylvestris* together with *Picea abies,* 41° 40' 22.8" N; 24° 26' 36.6" E. elev. 1756 m.
J. communis var. *saxatilis* - Bulgaria, *Adams Lab Acc. 13732-33, 14061-63,(Alex Tashev, 2012-JSI1-5),* Vitosha Region. Nature Park "Vitosha". Above the hut "Aleco" near the alpine timber line formed by a forest of *Picea abies*. On silicate rock together with *Vaccinium myrtillus, V. uliginosum, Ribes petraeum, Rubus idaeus, Calamagrostis arundinacea, Festuca valida (*Bulgarian endemic*)*, 42° 34' 52.1" N; 23° 17' 28.0" E. elev. 1848 m.
J. 'pygmaea' - Azerbaijan, shrubs, 0.5 - 1m tall, with *J. sabina*, on rocks in mountains. 41° 11.790' N; 48° 15.313' E. elev. 1649m *Adams Lab acc. 14321-14325* (*V. Farzaliyev 1-5*) 6 Jun 2014.

Exemplar specimens: *J. communis* var. *communis,* Stockholm, Sweden, *Adams 8167 (7846-7848); J. communis* var. *saxatilis,* Switzerland, *Adams 11164 (7618-7621)*. Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams,

Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from http://www.geneious.com/) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v. 3.1 (Ronquist and Huelsenbeck, 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

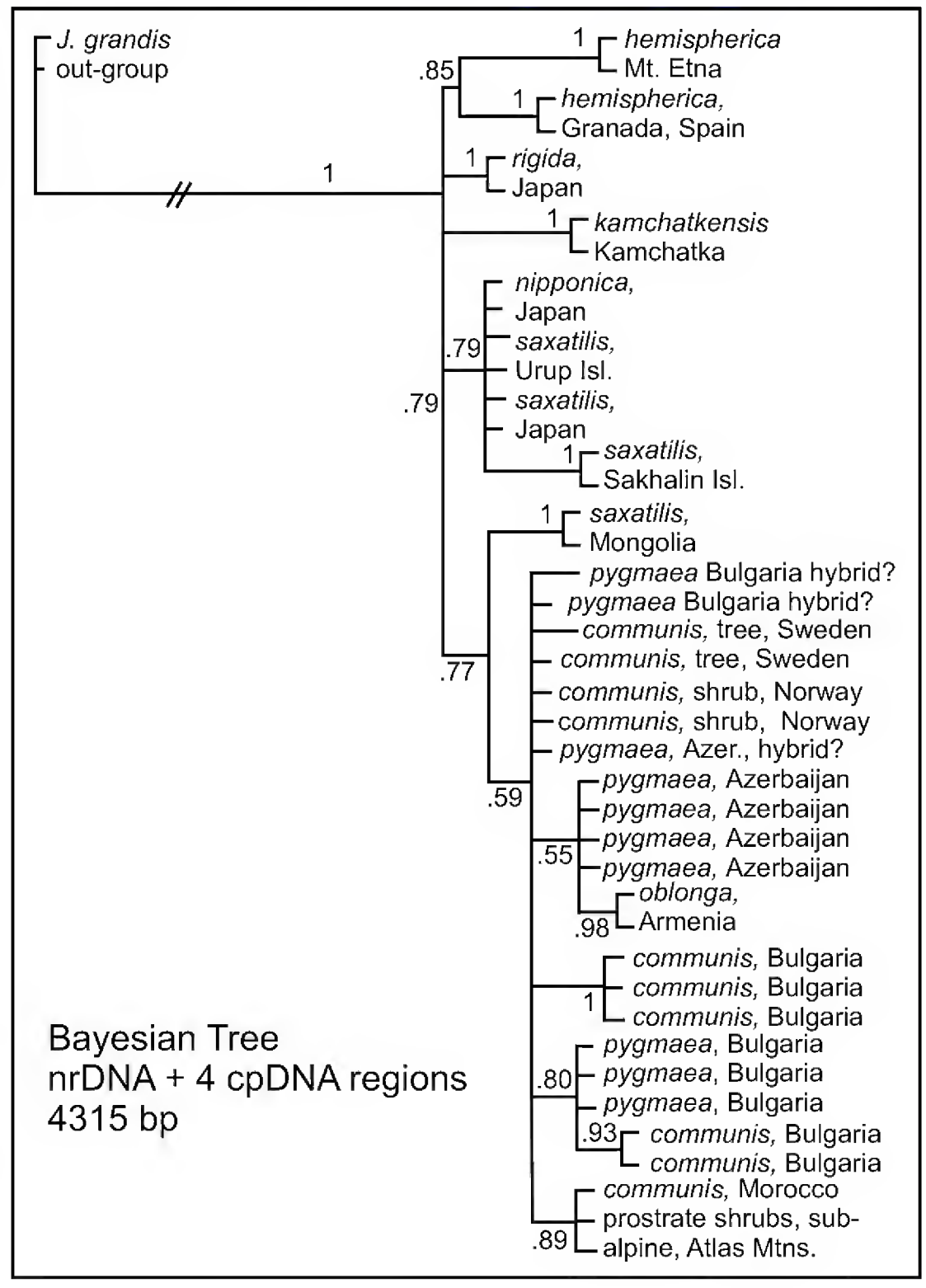

Figure 2. Bayesian tree of *Juniperus communis* taxa of the eastern hemisphere. Numbers at branch points are posterior probabilities. See text for discussion.

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS) and four cp-regions (petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF) yielded 4315 bp of data. The Bayesian consensus tree (Fig. 2) revealed the sub-alpine, prostrate *J. communis* plants of Morocco are in the clade with *J. communis* from Europe. They are not in the clade with var. *hemispherica* (Spain and Sicily). Our initial thought that these plants might be a southern extension of the plans in Spain was incorrect.

To examine the magnitude of the differences, a minimum spanning network was constructed (Fig. 3). *Juniperus communis*, eastern hemisphere, is divided into three groups: *J. communis*, Europe, *J. communis*, Japan and far east, and *J. c.* var. *hemispherica*, the latter divided among Mt. Etna, Sicily (type locality) and Sierra Nevada, Granada, Spain. All the samples of *J. 'pygmaea'* of Azerbaijan, are tightly grouped with *J. communis* from Europe (Fig. 3). Interestingly, the *J. communis 'oblonga'* of Armenia differs by 3 MEs (indels in this case) from *J. communis* of Sweden. The sub-alpine, prostrate *J. communis* plants of Morocco each only contained a single SNP and a one bp indel (deletion) in the trnSG sequence data for a total of 2 MEs (mutation events). The Moroccan prostrate *J. communis* appear to be a variant of *J. communis* of Europe and central Asia (Fig. 3) and not part of the var. *hemispherica* group.

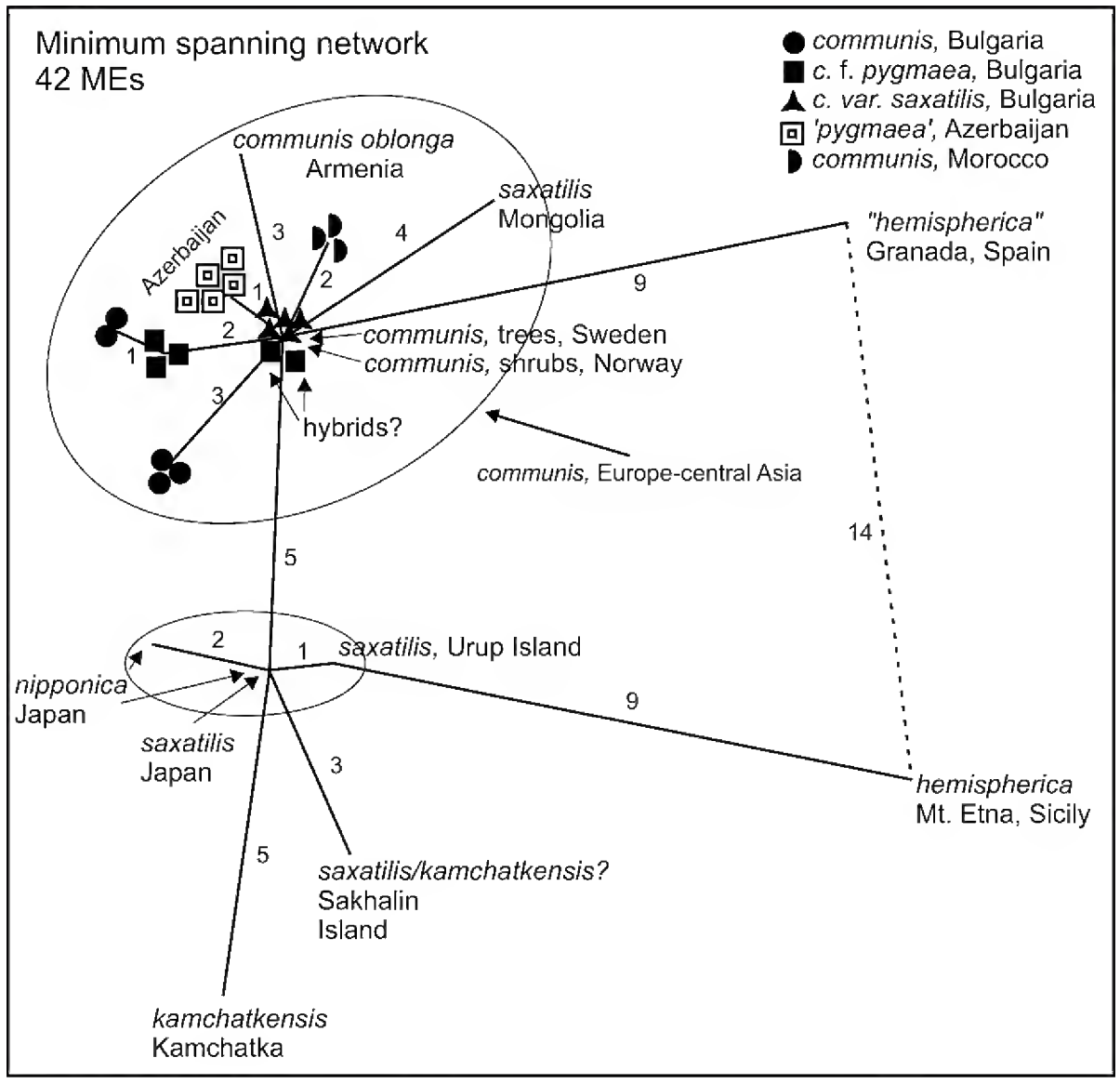

Figure 3. Minimum spanning network of *J. communis* and its varieties based on 42 MEs (mutational events = SNPs + indels). Numbers next to the links are the number of MEs. The dashed line is the second shortest link between the *hemispherica* taxa.

In summary, the sub-alpine, prostrate *J. communis* plants of Morocco do not appear, at this stage of research to be significantly different from *J. communis* of Europe to warrant it recognition as a distinct variety, in spite of the fact that typical *J. communis* are trees or shrub-trees. Transplant studies will likely be needed to ascertain if the prostrate habit is genetic or environmentally induced.

Clearly there considerable differences in habit of *J. communis* in various regions (Fig. 4) that may be due to only a few genes, as we are finding few DNA differences in our studies.

Figure 4. Plant habits of *J communis* f. *pygmaea*, Bulgaria, *J. communis 'oblonga',* Armenia compared to *J. 'pygmaea'*, Azerbaijan and *J. communis*, Spain and Hungary.

ACKNOWLEDGEMENTS

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A photographic essay on the development of seed cones in *Juniperus arizonica*

David Thornburg
2200 W. Winchester Lane, Cottonwood AZ 86326

and

Robert P. Adams
Biology Department, Baylor University, Waco, TX 76798 robert_adams@baylor.edu

ABSTRACT

During the course of observing pollination and seed cone development in local species of *Juniperus*, macro- and micro-photography was used to record changes in the external morphology of developing seed cones of *J. arizonica.*

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KEY WORDS: *Juniperus arizonica*, seed cone development, photographic essay.

As far as known, there are no chronological images the macro-development of seed cones in *J. arizonica* (Fig. 1). This photographic essay is presented to spur new research into the fertilization and development of seed cones in *Juniperus*.

Figure 1. mature, rose-colored seed cones of *J. arizonica.*

Ottley (1909) wrote the seminal paper on fertilization in *J. communis* and *J. virginiana.* However, that detailed study seems to have been scarcely followed up. Among her astute observations, she noted that fertilization in *J. communis* occurs about one year after pollination, but after about 1 to 2 months in *J. virginiana.* A photographic record of macro-development is important in the study of taxonomy of *Juniperus,* as it enables the observer to more clear distinguish male from female trees and to differentiate between immature normal cones and insect galls.

In the course of observing differences between seed cones (Adams, 2014), we have noticed the pattern of development in Arizona and herein present a photographic record of seed cone development in *J. arizonica.*

Pollen of *J. arizonica* was observed shedding from Jan to March, 2014 at this Arizona location. The first observed indication of pollination was observed and photographed on 20 Jan. 2014.

METHODS

From Jan 20, 2014 to Nov 5, 2014 photographs were taken on a single *J. arizonica* tree as its flowers matured to rose-red seed cones. The site is located about 3 miles SW of Cottonwood, Az, at Quail Springs Ranch, 34° 41' 16.458" N, 112° 03' 04.982" W, elevation 4090 ft. The photos were taken with a Sony digital camera, a digital microscope camera, and a 20X microscope.

RESULTS AND DISSCUSSION

The following photos (Fig. 2) document changes in size, shape and color of the developing seed cones. It is interesting that changes in color occur very quickly after the release of pollen in the area, implying fertilization is not long-delayed. Because Ottley (1909) reported that fertilization varied between 1-2 months (*J. virginiana*) to 1 year (*J. communis*), it is difficult to ascertain when fertilization is occurring in *J. arizonica*. It could be as long as a year after pollination, but because *J. arizonica* is phylogenetically closer to *J. virginiana* than *J. communis* (Adams, 2014), it may be that the time is also short (1-2 months) as in *J. virginiana*. In addition, Fechner (1976) argues that the shorter time from pollination fertilization in *J. virginiana* is correlated with the maturity of seeds in one year, versus 3 years for *J. communis* (with a time of one year between pollination and fertilization). Because the seed cones of *J. arizonica* mature in one year, their time from pollination to fertilization is likely short.

The development of seed cones may not be a sign of successful pollination. Fechner (1976) noted that in *J. virginiana* and *J. scopulorum* no differences were found in the size of filled and empty seeds. The deposition of pollen onto the stigma appears to trigger a mechanism which starts ovular development. Doroshenko (1928) theorized that the function of pollen in ovules is physical and that development could take place without fertilization. Fechner (1976) found that the germination of wind-pollinated seeds was lower than controlled-pollinated seeds from the same mother trees. He speculated this might be due to the presence of foreign pollen (eg. from another, nearby juniper species, such as cultivated *J. chinensis* and *J. sabina*). The alien juniper pollen could bring about seed development without a viable embryo. Recently, Adams, Thornburg and Corbet (2014) surveyed several *Juniperus* species for the incidence of empty vs filled seed cones. They reported *J. arizonica*, Cottonwood, AZ, had 34.4% (2010) and 33.4% (2011) filled cones. *Juniperus osteosperma* from nearby Sedona, AZ, had 79.0% (2010) and 7.2% (2011). They did not consider the fact that their *J. arizonica* population was nearly 100% *J. arizonica*, whereas the *J. osteosperma,* near Sedona, was growing near four other native juniper species, plus cultivated junipers nearby. The low incidence of filled seeds in 2011 (7.2%, *J. osteosperma*) might have been influenced by adjacent juniper pollen. In any case, it should be noted that our photos may well record seed cones with both filled and empty seeds.

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Figure 2. Photos of the development of seed cones of *J .arizonica* from 20 Jan. - 5 Nov. 2014.

Hymenopappus carrii (Asteraceae: Helenieae), a new species from the gulf coastal prairie of south-central Texas

Billie L. Turner
Plant Resources Center
The University of Texas
Austin TX 78712

ABSTRACT

A novel species, **Hymenopappus carrii**, is described from the coastal region of south-central Texas. It is closely related to **H. artemisiifolius**, but amply distinct in both vegetative and floral features.
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KEY WORDS: **Asteraceae, Helenieae**, *Hymenopappus*, Texas

The genus **Hymenopappus** was selected as a monographic study for my doctoral thesis (Turner 1956), this under the aegis of the late Marion Ownbey of Washington State University. The only addition to the Texas taxa since that publication has been **H. carrizoanus** (Turner, 1989). Gandhi & Thomas (Sida, Bot. Misc. 4: 113. 1989), however, reduced **H. artemisiifolius** to varietal status under their concept of **H. scabiosaeus** along with var. **riograndensis,** a view to which I do not subscribe, the two specific taxa being sympatric over a large portion of central Texas, showing little evidence of intergradation, except perhaps for the occasional hybridization.

The following novelty adds an additional species to the State's flora:

Hymenopappus carrii B.L. Turner, **sp. nov.**

Perennial (?) herbs, 30-45 cm high. **Roots,** slender, tap-rooted, but seemingly perennial. **Leaves** pinnately compound, forming a basal rosette, the blades 6-15 cm long, 3-5 cm wide, the ultimate divisions 2-3 mm wide, glabrous (on the type) or sparsely appressed-pubescent beneath (on the paratype; not at all tomentose-pubescent as in **H. artemisiifolius**); stem leaves 3-7, much-reduced upwards. **Capitulescence** a terminal cymose panicle of 10-20 heads ca 10 cm high, ca 6-8 cm across, the ultimate peduncles 1-2 cm long. **Heads** ca 1m high and as wide; involucral bracts 8-12, 1-2 seriate, obovate, 4-6 mm long, their apices broadly rounded with white-scarious apices 1-2 mm wide. **Receptacles** plane, 2-3 mm across. **Florets** 20-30 per head; corollas white, tubes ca 3 mm long, minutely glandular-pubescent; throats ca 1 mm long, glabrous or nearly so; lobes 5, reflexed, glabrous, ca 1mm long and as wide. **Stamens** with yellow anthers ca 2 mm long, their apical appendages glandular. **Achenes** 4-sided, ca 3 mm long, 1 mm wide, pubescent throughout with minute ciliate hairs mostly 0.2 mm long or less; pappus a ring of minute membranous scales 0.1-0.2 mm high.

HOLOTYPE: **TEXAS. MATAGORDA CO.:** "rare on preserve (at least in S half), noted only in salty prairie grassland on somewhat tight slightly saline clay on +/- level topography about 1 m above salt marsh along Mad Island Lake, !.7 airmiles NE of HQ of Mad Island Marsh Preserve," 28 39 45.6 N, 96 05 23.7 W, elev. 2-3 m, 20 Apr 2004, *W.R. Carr 23034* (TEX).

ADDITIONAL SPECIMEN EXAMINED: **TEXAS. REFUGIO CO.:** "coastal prairie, off hwy. 35 at intersection of 774," 11 Apr 1963, *C.L. Lundell & A.A. Lundell 17388* (LL).

I first came to know this taxon by the Refugio Co. collection cited above, thinking I might give it varietal status, but instead included it within my concept (at the time) of **H. artemisiifolius** var.

riograndensis. The subsequent collection by Carr strongly suggests that there is a population of nearly glabrous plants (cf. Fig. 2) having markedly pinnatifid leaves and minutely ciliate achenes with reduced pappus scales that occur only along the tidal slopes of south-central Texas along the Gulf of Mexico (Matagorda and Refugio counties). The roots of both collections differ markedly from those **H. artemisiifolius**, the latter being enlarged and clearly tap-rooted, as opposed to the very slender, branched roots of the **H. carrii**. It is likely that additional populations of the novelty will be found in similar habitats of the area concerned.

It is a pleasure to name the species for my long time colleague and extraordinary botanist, William R. Carr, collector of the Type specimen.

The following key to the Texas taxa of **Hymenopappus** should prove helpful:

1. Ray florets white, prominent; trans-Pecos, Guadalupe Mountains...**H. biennis**
1. Ray florets absent...(**2**)

2. Perennial plants with woody or lignescent rootstocks. ..... **H. filifolius**
2. Annual, biennial or weakly perennial plants, with herbaceous taproots...(**3**)

3. Corollas yellow. ..... **H. flavescens**
3. Corollas white, creamy-white or purplish-tinged...(**4**)

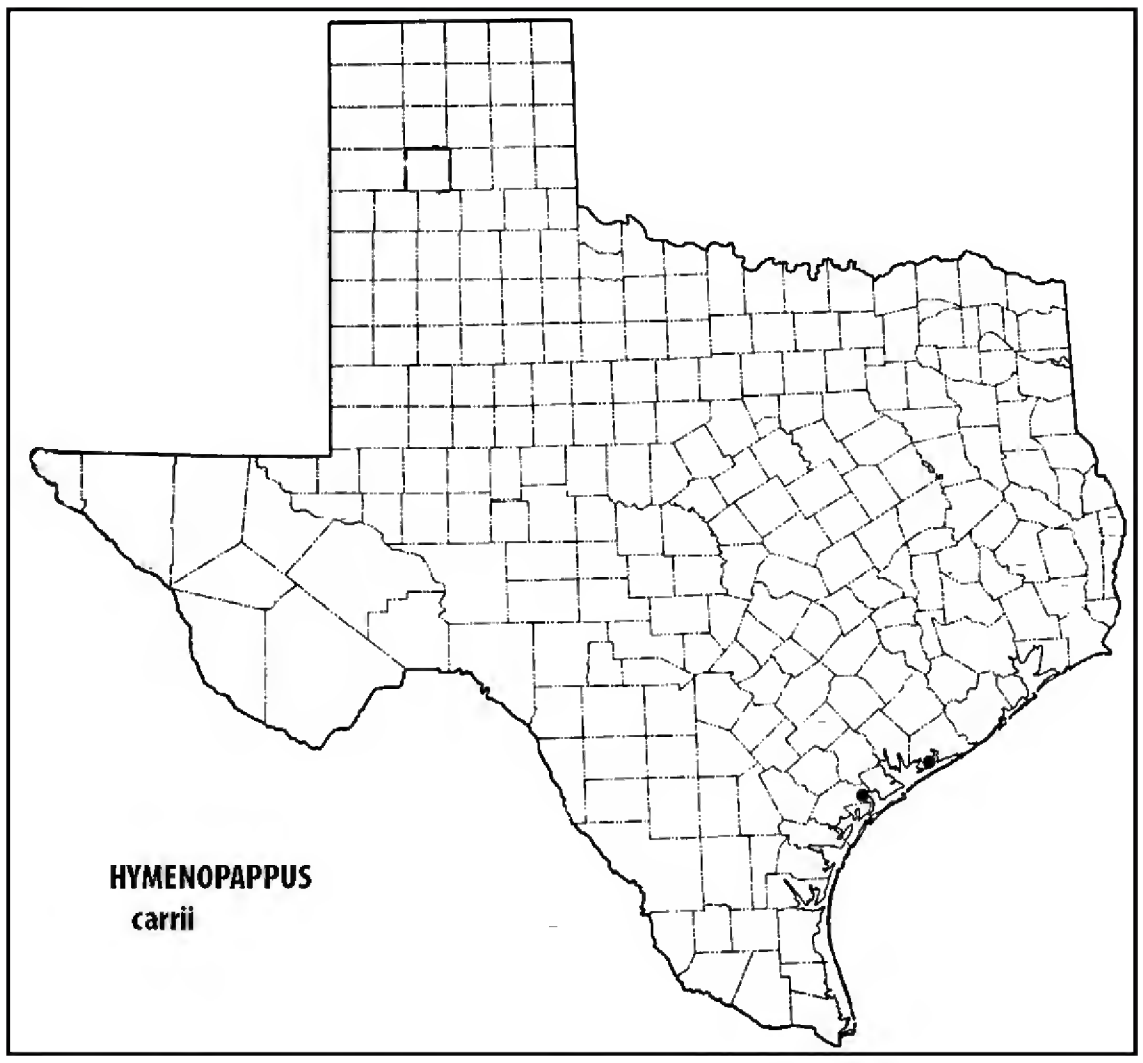
4. Leaves entire to merely lobed or dissected, the ultimate divisions mostly 2-6 mm wide...(**6**)
4. Leaves dissected with linear divisions, the latter mostly 0.5-1.5 mm wide...(**5**)

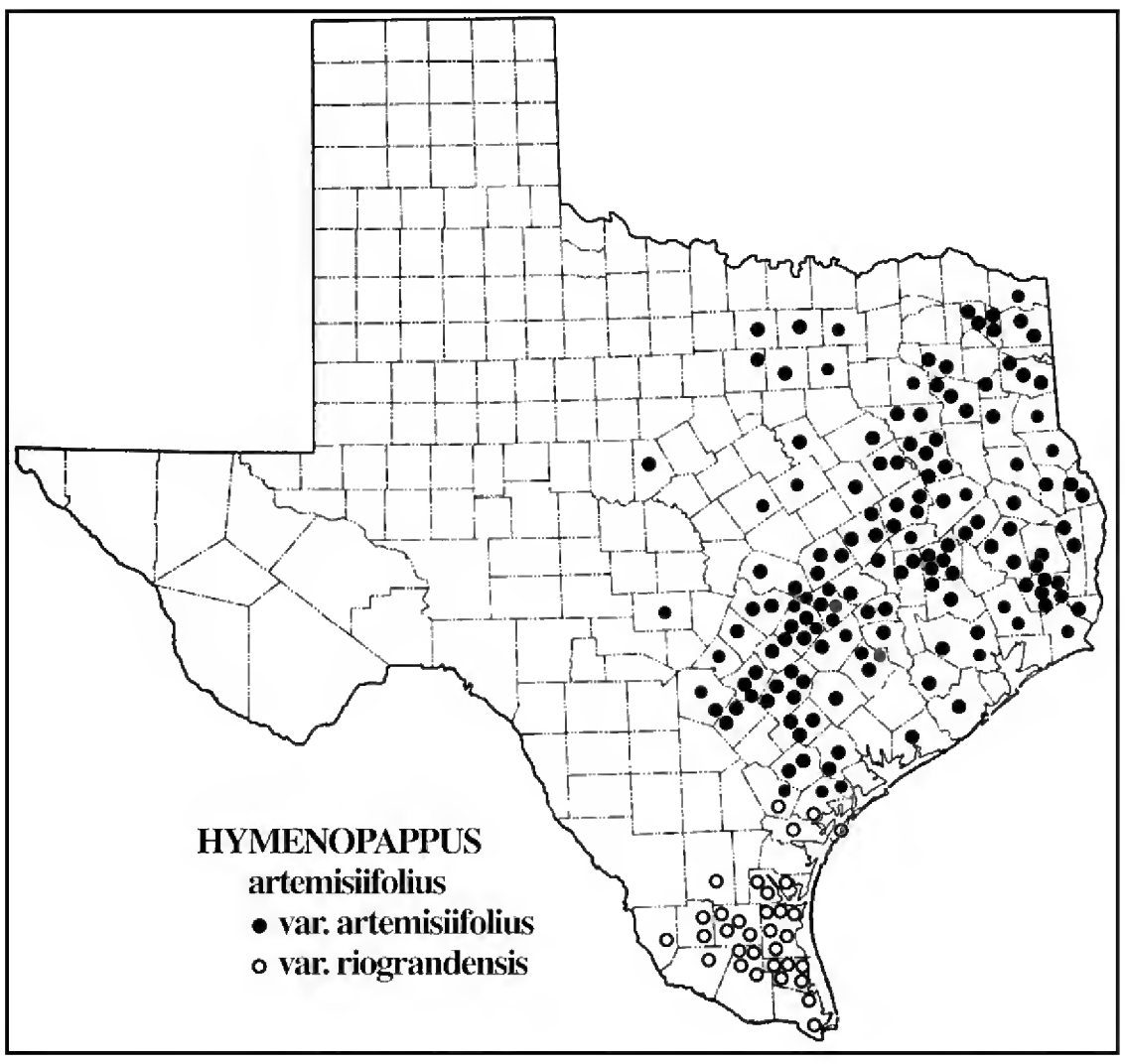
5. Stems about equally leafy throughout, the leaves not much reduced upwards; involucres tomentulose throughout; corolla throat campanulate.....**H. carrizoanus**
5. Stems mostly leafy below, the leaves much reduced upwards; involucres variously pubescent to glabrate, corolla throat campanulate....**H. tenuifolius**

6. Under surfaces of leaves glabrous or nearly so; pappus scales 0.1-0.2 mm long; saline sandy soils along Gulf of Mexico .....**H. carrii**
6. Under surfaces of leaves densely velvety white-pubescent; pappus scales 0.4-2.0 mm long (rarely not); mostly interior plants of central and southern Texas...(**7**)

7. Leaves of basal rosette pinnately to bipinnately dissected; florets with white corollas; mostly clayey soils.....**H. scabiosaeus**
7. Leaves of basal rosette simple to deeply lobed; floret corollas variously rosy-vinaceous or purplish-colored; mostly sandy soils...**H. artemisiifolius**

[*H. scabiosaeus* is represented in Texas by the var. *corymbosus*; *H. artemisiifolius* by var. *artemisiifolius* and var. *riograndensis*, as shown in the maps below, these taken from my latest, up-dated, Atlas of the Vascular Plants of Texas (Turner, 2003)]


HYMENOPAPPUS
carrii


HYMENOPAPPUS
artemisiifolius
● var. artemisiifolius
○ var. riograndensis

ACKNOWLEDGEMENTS

Thanks to my colleague, Bill Carr, for information regarding the ecological status of the type locality, and the photo of type material. Jana Kos kindly provided editorial input.

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Fig. 1. Holotype of *Hymenopappus carrii*.

Fig. 2. Capitulescence (top photo) and basal rosette (lower photo) of *H. carrii* (from the Type itself, just before pressing).

Lectotypification of *Viscum latifolium* Lamarck

Job Kuijt
649 Lost Lake Road, Victoria, BC V9B 6E3, Canada
jobkuijt@uvic.ca

ABSTRACT

Viscum latifolium Lamarck is lectotypified. Published on-line **www.phytologia.org** *Phytologia 97(2): 137-138 (April 1, 2015)*. ISSN 030319430.

KEY WORDS: *Viscum latifolium*, *Phoradendron racemosum*, *Passovia pyrifolia*, lectotypification.

The name *Viscum latifolium* has appeared at least three times in the botanical literature, the earliest being published by Lamarck (1789). In the early monograph of the viscaceous genus *Phoradendron* (Trelease 1916) as well as in my recent one (Kuijt 2003), *Viscum latifolium* Lam. was listed as a synonym of *P. racemosum* (Aubl.) Krug & Urban. Trelease neither typified the species nor listed a Lamarck specimen under *Phoradendron racemosum*. In my own monograph, *Stoupy s.n.* (P-LAM) was incorrectly listed as the holotype of *V. latifolium* Lam. There exists an urgent reason to correct this error, as explained below.

The relevant statement following Lamarck's description of *V. latifolium* reads as follows: "Ce *Gui* croît dans l'Isle de St. Dominique. Nous en possédont un bel exemplaire rapporté de Cayenne par M. Stoupy …". No collector was mentioned for the Hispaniola material. In other words, he based his description on an unnamed specimen from Hispaniola and reported that he also possessed a good specimen from Cayenne collected by Stoupy. The former would have been the holotype but is not present in P-LAM. Since no collector or other data are provided, it would be impossible to prove that any other specimen can be regarded as the holotype. At first sight, therefore, it would be tempting to typify Lamarck's name with the Stoupy specimen, which is in excellent condition and is present in P-LAM. However, this would be a serious mistake, for that specimen unequivocally belongs to what is presently known as *Passovia pyrifolia* (Kunth) Tiegh. (Loranthaceae), which does not occur on any of the Greater Antilles except Jamaica. In fact, that species is the most common and widespread species of continental neotropical Loranthaceae, and the change of its name that would result from its designation as the type of *V. latifolium* would be extremely unfortunate.

Fortunately, this change is unnecessary. As Dr. Kanchi Gandhi (GH) has kindly pointed out, Lamarck also refers to a plate in Plumier's "Plantarum americanarum …" (Plumier 1760, tab. CCLVIII, Fig. 4). This figure, labeled "*Viscum folius ovatis*", undoubtedly corresponds to the present *Phoradendron racemosum*. In consequence, I herewith designate that figure as lectotype of *Viscum latifolium* Lamarck. This lectotypification has the double effect of retroactively justifying the synonymy of Lamarck's name as listed by Trelease (1916) and myself (Kuijt 2003) and, most importantly, avoiding a calamitous name change of *Passovia pyrifolia*. It is clear that Lamarck was in error in considering his two specimens to be the same species.

ACKNOWLEDGEMENTS

The nomenclatural advice of Dr. Kanchi Gandhi (GH) is gratefully acknowledged.

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Endemism in two new species of *Dendrophthora* (Viscaceae) from Cerro Jefe, Panama

Job Kuijt
649 Lost Lake Road, Victoria, BC V9B 6E3, Canada
jobkuijt@uvic.ca

and

Jerry & Linda Harrison
PTY 9617, 2250 NW 114th Ave., Unit 1P, Miami, FL 33172, USA
jharrison@canopytower.com

ABSTRACT

Two new species, **Dendrophthora fortis** J. Kuijt, **sp. nov.** and **D. perlicarpa** J. Kuijt, **sp. nov.**, are described and illustrated from the Cerro Jefe area, Panama. At least *D. fortis* is distinctive in having a dichotomous branching habit, the apex aborting following the formation of one pair of leaves. *D. fortis* is related to *D. obliqua* with which it has been confused in the past. The two species are known only from the Cerro Jefe area except for *D. fortis*, of which there are two records from El Valle de Antón. Published on-line **www.phytologia.org** *Phytologia 97(2): 139-144 (April 1, 2015).* ISSN 030319430.

KEY WORDS: *Dendrophthora fortis*, *D. perlicarpa,* Viscaceae, Cerro Jefe, endemism.

The large neotropical genus *Dendrophthora* (Viscaceae) includes an assemblage of species of strikingly robust habit, its only well known species being *D. obliqua* (Presl) Wiens. The group ranges from Peru to Venezuela and Panama and, like nearly all continental congeners, prefers high altitudes. Until fairly recently, all of the species of this group were thought to belong to the closely allied genus *Phoradendron*. The documented presence of unilocular anthers of several entities has led to their transfer to *Dendrophthora*.

A prominent structural feature of *D. obliqua* and several others is a dichotomous habit in which each innovation bears one pair of distal leaves, below which stand several pairs of large cataphylls. The apex of the innovation aborts, and new innovations emerge in the axils of the foliage leaves. Inflorescences also develop from the foliar nodes (See the illustration in Kuijt 1986, Fig. 43).

The taxonomic problems in studying this assemblage are several. With the possible exception of *D. obliqua* and one of the species here introduced, the species are poorly represented in major herbaria and, due to their coarseness and rigidity, are often in fragmentary condition. Fruit color and shape, which sometimes are of pivotal taxonomic value, are not ascertainable from herbarium specimens and are only rarely included in label information. Male and female flowers are similar and, consequently, the distribution of the sexes is often not clear. Confusingly, some entities appear to combine both dichotomous and percurrent habits. Several entities described in the past inhabit inaccessible areas such as the summits of tepuis.

An attempt was made earlier (Kuijt, in Kuijt & Kellogg 1996) to analyze the complex, but this attempt will need revision if and when more material becomes available. Our present contribution focuses on the northernmost elements as they occur in Panama, almost entirely in the Cerro Jefe area. The collections in the past from this mountain range have mostly been identified as *D. obliqua*. Our recent field and herbarium studies have established, however, not only that *D. obliqua* does not occur in Panama

but also, surprisingly, that two different undescribed species of the assemblage are present on Cerro Jefe, at least *D. fortis* with a strictly dichotomous habit. It is the purpose of our contribution to describe and illustrate these new species.

DENDROPHTHORA FORTIS J. Kuijt, **sp. nov. Figs. 1 & 2.**

Diagnosis Plants bright green, leaf blades symmetrical, elliptical, flat, shiny when fresh, apex rounded; venation palmate, with 8--10 veins, evident; dioecious. Inflorescence with mostly 6 fertile internodes, flowers 9--12 per fertile bract. Fruits globular, 3 mm in diameter, light pink.

Description **Very stout, glabrous, bright green plants**, dichotomous by abortion of the shoot apex after forming one pair of foliage leaves; innovations with 3 or 4 pairs of blunt cataphylls, the basal ones ca. 4 mm above the base, the upper ones about halfway to the foliage leaves. **Internodes terete**, 5--20 cm long, nodes somewhat swollen, to 2 cm thick in age. **Foliage leaves** coriaceous, shiny when fresh, with blades 7--15 cm long and 6--10 cm broad, ovate-elliptical to nearly orbicular, symmetrical, petiole massive, flat, 6--7 mm, often red, base of blade mostly obtuse, apex rounded, venation palmate, the 8--10 veins very prominent and reddish when fresh, margins leathery, brown. Dioecious, female plants predominating. **Female inflorescence** to 9 cm long, turning reddish or orange in age, consisting of a short (6--7 mm) double peduncle followed by (3--5) 6 fertile internodes each ca. 10 mm long, the apical ones shortest; fertile internodes slightly swollen, flowers triseriate, somewhat sunken in the rachis, 9--12 above each fertile bract, the terminal internode with as few as 3 flowers. **Male inflorescence** of similar size and construction, also triseriate, flowers ca. 18 per fertile bract. **Fruits** 3 mm in diameter, globular, light pink.

TYPE: **PANAMA**. **PANAMÁ**: ca. 23 km from turn off to Los Altos de Cerro Azul from "Fucer," reached from town of "24 Diciembre" off Inter-American Hwy.; station along road about halfway between Cerro Jefe towers and Vistamares overlook; cloud forest, 900 m, 09°14'6.45"N, 79°23'10.92"W, on *Clusia* sp., 28 Jun 2014, *J. & L. Harrison 639* (Holotype UCH; Isotypes PMA, US).

In general aspect *Dendrophthora fortis* is similar to, and probably closely related to, *D. obliqua*, which is known especially from Ecuador. Both species (and a number of other species in *Dendrophthora* and the related genus *Phoradendron*) demonstrate a consistently dichotomous habit characterized by the abortion of the apex of the innovation. The inflorescences are developed especially on older nodes, often in clusters. *D. fortis* differs from *D. obliqua* most obviously in its elliptical, symmetrical, round-tipped leaf blades with red veins and petioles, while *D. obliqua* leaves at least at maturity have a characteristically asymmetrical shape and acute apex. *D. obliqua* has 20--40 flowers per fertile bract, but the number is about half that in *D. fortis*. *D. obliqua* is monoecious, the sexes said to be on separate spikes at least in Ecuador, but *D. fortis* is dioecious. Curiously, out of the 22 collections studied, 20 were female and only 2 were male; nevertheless, nearly every female flower appears to develop into a fruit, suggesting apogamy.

The name *Dendrophthora obliqua* has, in the past, mistakenly been applied to Mesoamerican plants, including some from Cerro Jefe (Kuijt 1978, 1990), partly because of the confusion with a species of *Phoradendron* of similar branching habit (*P. nitens* Kuijt; Kuijt, 1964: 275, 316). The possibility suggested in Kuijt (1990: 136) that the Cerro Jefe material is taxonomically distinct from *D. obliqua* is thus confirmed. The true *D. obliqua* is probably limited to the northern Andean region.

Dendrophthora fortis appears to be an endemic to Cerro Jefe except for a localized population in the El Valle area (*Churchill 3925*, *3926*, *Dwyer10556*). In fact, the citations below show *D. fortis* to be limited to a small area on the mountain; the majority of known collections are from essentially the same location. Cerro Jefe is well known for its high degree of endemism (De Sedas M. et al. 2010). Of the 1260

species thus far believed to be endemic to Panama, 222 (ca. 18%) are present on Cerro Jefe and 66 (5.2%) are limited to the locality.

Etymology: The epithet "*fortis*" refers to the stout structure of the plant.

ADDITIONAL SPECIMENS EXAMINED: **PANAMA**. **COCLÉ**: Area of El Valle, 2 km E of La Mesa, N slope of Cerro Gaital, dwarf *Clusia* forest, 8°38'N, 80°7'W, on Guttiferae, 800 m, 24 Nov 1983, *H.W. Churchill 3925* (MO), *3926* (MO); 8.4 km from village of El Valle, vicinity of La Mesa, 30 Mar 1973, *J.D. Dwyer 10556* (MO). **PANAMÁ**: 200 m de la torre de telecomunicaciones en Cerro Jefe, 09°13'13.0"N, 079°22'28.4"W, 17 Nov 2003, on *Clusia*, *FLORPLAN, De Gracia, Martínez, Cabrales-Alin, Burman 6406* (PMA); summit of Cerro Jefe and along road on E slope, 9°15'N, 79°30'W, 900--1000 m, 5 Apr 1982, *S. Knapp & M. Huft 4581* (MO); Cerro Jefe: on *Clusia*, 12 Sep 1985, *L. Carrasquilla 2125* (MO); 1000 m, 21 Sep 1986, on *Clusia*, *Valdespino & Aranda 147* (MO, PMA); near tower, 2400 ft, 23 May 1980, *Antonio 4724* (MO, PMA); trail leading west from summit, 24 Sep 1975, *J.T. & F. Witherspoon 8505* (MO); in *Clusia* forest, 2700--3000 ft, 27 Jan 1966, *E.L. Tyson, J. Dwyer, & K. Blum 3193* (MO), *3282* (MO, 2x); cloud forest dominated by *Clusia* & *Colpothrynax cookii*, 1000 m, 14 Jul 1975, *S. Mori 7123* (MO); along trail on ridge running NE from summit, 1000 m, 18 Dec 1974, *S. Mori, J. Kallunki, and 5 others 3766* (MO); along trail on ridge running NE from summit, 1000 m, on *Clusia*, *S. Mori & 5 others: Cochrane, Hansen, Kowal, & Nee 3766* (MO); forested slopes near radio tower, 950 m, 9°15'N, 79°30'W, 11 Oct 1985, *G. McPherson 7141* (MO); E slope, dirt track near radio tower, 9°15'N, 79°30'W, 950--1000 m, *S. Knapp & J. Mallet 5181* (MO, UC); road N from summit, 9°14'N, 79°23'W, 20 Jan 1984, *H.W. Churchill 4307* (MO); summit, 900--1000 m, 4 Apr 1982, *M. Huft & S. Knapp 1720* (MO); summit near radio towers, dwarf *Clusia* forest, on *Clusia*, 9°4'N, 79°23'W, 1000 m, *W.H. Churchill 3947* (MO); 800--1000 m, 23 Feb 1977, *J.P. Folsom, R. Lantz, & J. Atwood 1856* (MO); *Clusia* forest, 2700--3000 ft, 27 Jan 1966, *E.L. Tyson, J. Dwyer, & K. Blum 3193* (MO), *3282* (MO, 2x); top of Cerro Jefe, at tower, 950 m, 22 May 1980, *J.P. Folsom, J.D. Mauseth, & T. Antonio 7800* (MO); 2700--3000 ft, 9 Jul 1966, *E.L. Tyson, J. Dwyer, & K. Blum 4340* (MO).

DENDROPHTHORA PERLICARPA J. Kuijt, **sp. nov. Fig. 3 & 4.**

Diagnosis. Bright green, dichotomous and percurrent plants, leaves ovate, standing sideways, flat and coriaceous, shiny, apex ca. acute, venation palmate but obscure when fresh; dioecious. Female inflorescence 3--5 cm long, with 2 or 3 sterile basal internodes followed by 5 or 6 fertile internodes each with 6 or 7 flowers in triseriate pattern. Fruit 5--6 mm in diameter, spherical, pearly white.

Description. **Bright green, dichotomous and percurrent plants**, each innovation apically aborting and bearing a single pair of leaves or terminating in an inflorescence. **Internodes stout, terete**, smooth; innovations bearing 3--5 pairs of obtuse basal cataphylls, the uppermost pair placed halfway or slightly lower to the foliar node above; similar intercalary cataphylls present on percurrent shoots. **Leaf blades** 5--7 cm long, 3.5--5 cm wide, ovate, shiny, coriaceous, apex acute or nearly so, base obtuse, petiole 3 mm long, stout, the youngest leaves almost sessile; venation palmate but obscure when fresh, with 3 or 5 veins. Leaf margin brown, often irregularly granular when young. Dioecious, only the female known. **Female inflorescence** 3--5 cm long at early anthesis, elongating slightly in fruit, each with 2(3) sterile internodes basally, these followed by 5 or 6 fertile internodes. Flowers triseriate, 6 or 7 per fertile bract on the larger internodes, at first deeply immersed in the swollen rachis, whitish, red inside, the adjacent internodal area red. **Fruit** spherical, 5--6 mm in diameter, clear pearly white, the petals half closed, brown, lightly tomentose also on the adjacent part of the fruit.

TYPE. **PANAMA. PANAMÁ:** Los Altos de Cerro Azul, close to Cerro Jefe, midway along El Cantar trail near first rain shelter (starting from El Fortin trail head at end of Calle Andorra), premontane forest, ~ 850 m, 9°13'50.938"N, 79°24'10.493"W, 23 Jan 2015, *J. & L. Harrison 680* (Holotype: UCH).

The leaves of *Dendrophthora perlicarpa* provide the most obvious contrasts with those of the only other known Cerro Jefe congener, *D. fortis*. The leaf blades of the former are ovate, with an acute or nearly acute apex and an obscure major venation of 3--5 veins, while those of *D. fortis* are mostly elliptical or nearly so, with rounded apices, and showing 8--10 strongly marked veins. Additional differences are seen in the female inflorescences that in *D. perlicarpa* bear 6 or 7 flowers per fertile bract, but in *D. fortis* 9--12. Finally, the white fruits of *D. perlicarpa*, being 5--6 mm in diameter, contrast with those of *D. fortis* that are 3 mm in diameter and light pink.

Because only a single specimen was collected (a much younger plant was seen nearby), it is not certain that the unusual terminal inflorescences here reported are standard equipment in *D. perlicarpa*. No other leafy Mesoamerican species of the genus are known to have terminal inflorescences. Its unusual floral color pattern may be unique in the genus generally.

Etymology. The epithet "*perlicarpa*" refers to the pearly luster of the fruit.

DISCUSSION

In contrast to most previously described elements of the assemblage, the sex distribution of both new species is clearly of a dioecious type. The only specimen available of *D. perlicarpa* bears fruits or female flowers, or the remainder of their infructescences demonstrates large floral cavities in which fruits have been present in the past, documenting its dioecious status. The situation in *D. fortis* initially was puzzling because only female specimens seemed available. Eventually, however, two purely male specimens were located, as listed above, so that we can be certain that this species is also dioecious. Curiously, however, the number of female specimens we have studied greatly outnumbers the male ones (20 to 2). Infructescences regularly bear fruits in all positions, a feature that raises the possibility of apogamy. One of the male specimens of *D. fortis* (*Knapp & Mallet 5181*, UC) has previously been documented to have unilocular anthers, confirming its generic status.

ACKNOWLEDGEMENTS

We are obliged to Roy Gereau (MO) for nomenclatural advice.

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Fig. 1. General habit of the type of *Dendrophthora fortis*.

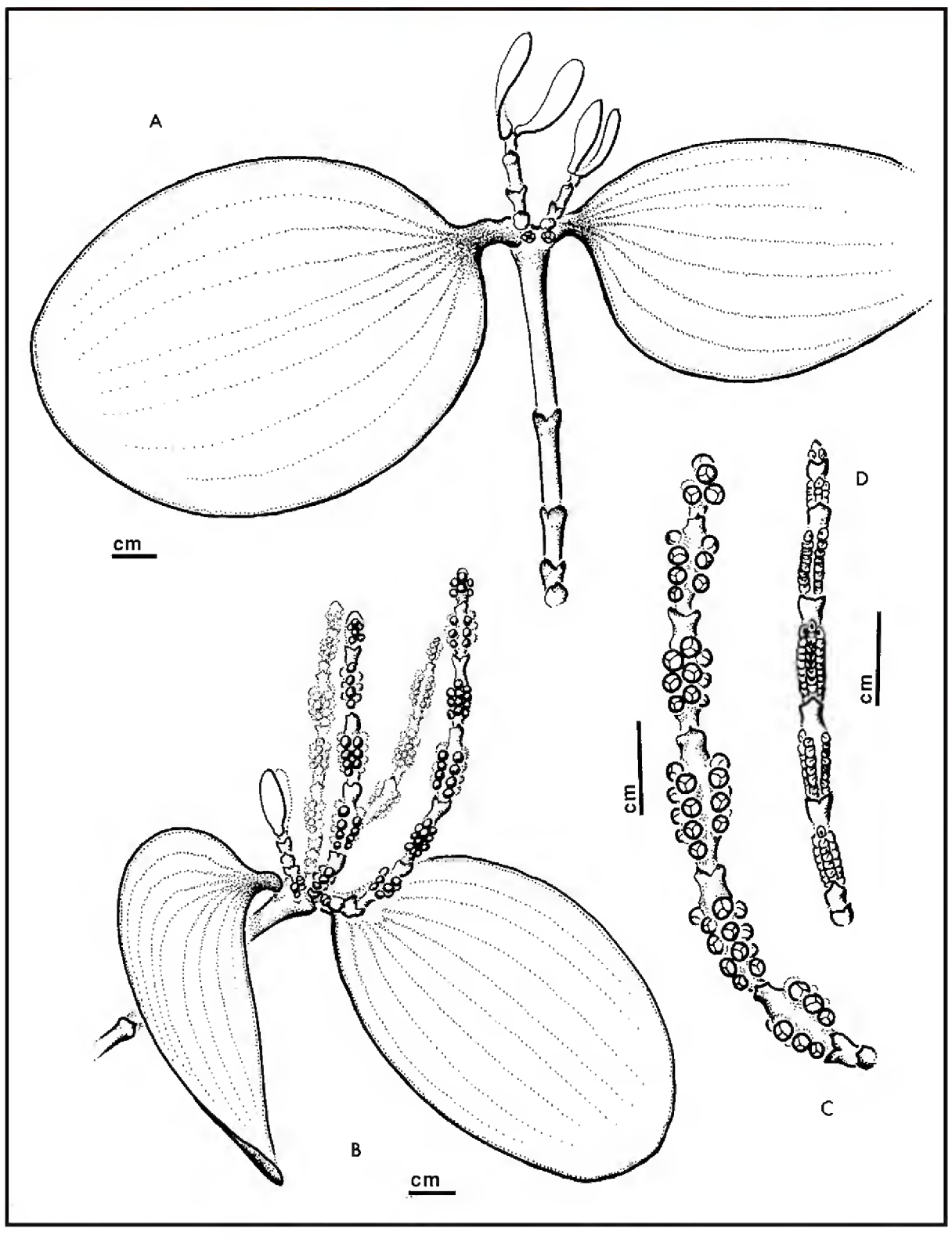

Fig. 2. *Dendrophthora fortis.* A--C: *Harrison & Harrison 639* (UCH). D: *Knapp & Mallet 5181* (MO). A. Young innovation. B. Innovation bearing inflorescences and young shoot. C. Female inflorescence. D. Male inflorescence.

Fig. 3. Flowers and inflorescences of the type of *Dendrophthora perlicarpa*.

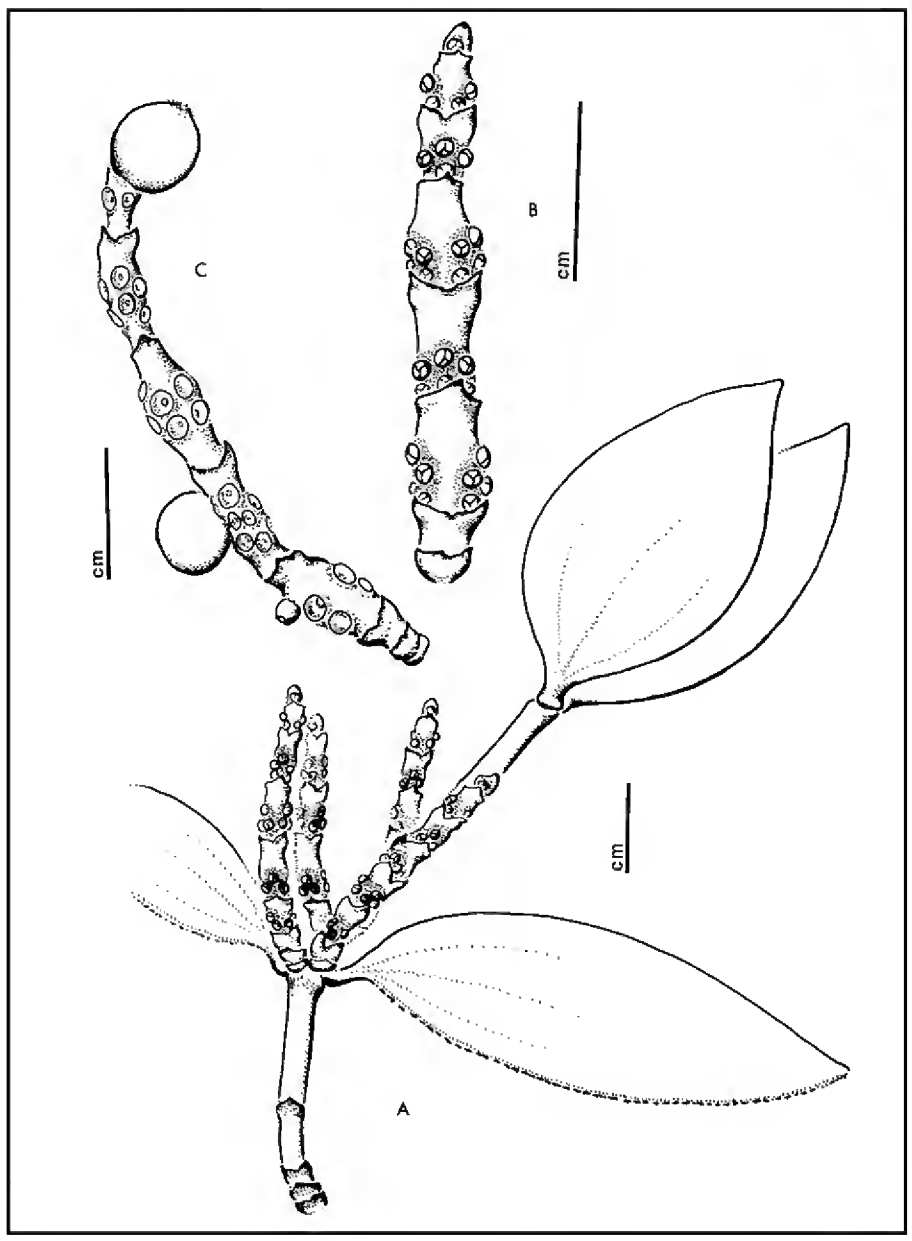

Fig. 4. *Dendrophthora perlicarpa*. *Harrison & Harrison 680* (UCH)**.** A. Habit. B. Young female inflorescence. C. Infructescence.

The volatile leaf oils of *Juniperus flaccida* Schltdl., *J. martinezii* Pérez de la Rosa and *J. poblana* (Mart.) R. P. Adams, re-examined

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA Robert_Adams@baylor.edu

and

Thomas A. Zanoni
New York Botanical Garden, 200th Street at Southern Blvd.
Bronx, NY 10458-5126

ABSTRACT

The volatile leaf oils of *J. flaccida, J. martinezii and J. poblana* are re-examined and the composition is published using FID quantitation. The leaf oil of *J. flaccida* is dominated by α-pinene (65.0%) with moderate amounts of β-pinene (4.8%), myrcene (4.3%), limonene (3.5%), β-phellandrene (3.4%), linalool (2.9%) and manool oxide (3.5%). The oil of *J. poblana* is somewhat similar as it is dominated by α-pinene (52.9%) with moderate amounts of β-pinene (4.2%), myrcene (4.3%), limonene (2.2%), β-phellandrene (3.5%) and linalool (1.9%), but contains several unique compounds: δ-2-carene (1.8%), δ-3-carene (1.4%), trans-verbenol (2.7%), methyl chavicol (0.7%), and (E)-nerolidol (2.5%). The oil of *J. martinezii* was quite distinct with major components being α-pinene (16.6%), sabinene (10.4%) and camphor (11.1%) and moderate amounts of β-pinene (1.4%), myrcene (3.6%), limonene (1.8%), β-phellandrene (5.3%),linalool (2.8%), γ-terpinene (1.8%) and terpinen-4-ol (6.1%). It also contain several unique compounds: p-cymenene (0.7%), karahanaenone (1.3%), trans-dehydrocarvone (0.6%), trans-chrysanthenyl acetate (0.5%), linalool acetate (0.4%), noe0iso-3-thyjanyl acetate (0.8%), an aromatic phenol (KI 1320, 0.5%), trans-muurola-4(14), 5-diene (0.7%), epi-cubebol (0.5%), cubebol (1.1%), 1-epi-cubebol (1.0%), and an unknown diterpene (KI 1978, 0.6%). Published on-line **www.phytologia.org** *Phytologia 97(2): 145-151 (April 1, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus flaccida, J. martinezii, J. poblana, Cupressaceae*, terpenes, leaf essential oil.

The taxonomy of *Juniperus flaccida* Schltdl. has been somewhat unsettled. Generally, flaccid (weeping) foliaged junipers in Mexico (and the Chisos Mtns., Texas) have been referred to as *J. flaccida*. The first systematic treatment of these junipers was by Martinez (1963) who recognized two varieties: *J. f.* var. *typica* and *J. f.* var. *poblana* Mart. *Juniperus f.* var. *flaccida* has large seed cones (9-20 mm), with 6-10 seeds, and pendulous (flaccid) foliage and branchlets. *Juniperus f.* var. *poblana* also has large seed cones (9-12 mm), with 6-10 seeds, but the foliage is distichous and in vertical planes like *Thuja*, and not very flaccid (Zanoni and Adams, 1976, 1979; Adams, 2014). Pérez de la Rosa (1985) discovered a population of trees that had small seed cones (5-7 mm), with 1-2 seeds per cone and with foliage somewhat drooping but branchlets erect. He described this taxon as a new species, *J. martinezii* Pérez de la Rosa. Except for the seed cones, the taxon looks similar to *J. flaccida*; indeed Silba (1985) treated it as *J. flaccida* var. *martinezii* (Pérez de la Rosa) Silba. Each of these varieties has leaf margins that are hyaline and nearly entire, with either a few small teeth or merely a wavy margin (Adams, 2014). However, they are considered part of the serrate leaf margined *Juniperus* species of the western hemisphere (Adams, 2014).

Adams et al. (1990) compared the leaf essential oils and found considerable differences among the *J. flaccida* varieties. However, they decided to accept *J. flaccida* var. *martinezii* until "...additional data, such as from DNA analysis, are available." (Adams et al. 1990).

DNA sequencing of nrDNA (ITS) and trnC-trnD (Adams, et al. 2006) revealed that *J. flaccida* varieties are not monophyletic and they recognized *J. martinezii* and *J. f.* var. *poblana* as *J. poblana* (Mart.) R. P. Adams. More recently, Adams and Schwarzbach (2013) published a detailed phylogeny of the serrate junipers of the western hemisphere based on nrDNA and four cp genes. They found *J. flaccida* (var. *flaccida*) in a clade with *J. standleyi* (Fig. 1) and *J. poblana* (*J. f.* var. *poblana*) in a well supported sister clade. *Juniperus martinezii* (*J. f.* var. *martinezii*) was found in a well supported clade with *J. durangensis* (Fig. 1). Their work appears to solidify support for the recognition of *J. martinezii* and *J. poblana.*

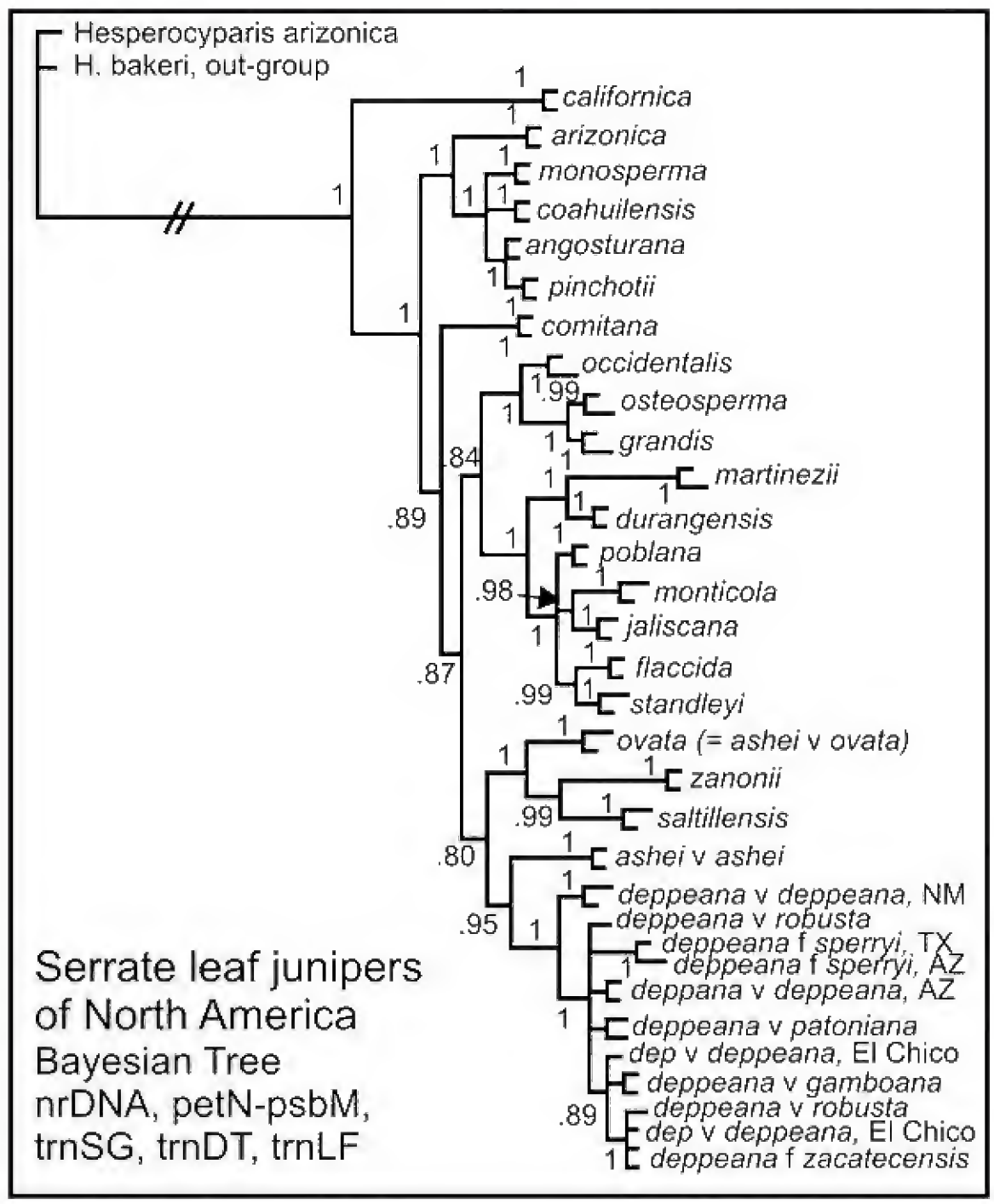

Figure 1. Bayesian analysis of the serrate leaf junipers of North America. Numbers are posterior probabilities. From Adams and Schwarzbach (2013). See text for discussion

The composition of the volatile leaf oils of *J. flaccida* and *J. f.* var. *poblana* were first reported by Adams, Zanoni and Hogge (1984). The composition of the leaf oil of *J. martinezii* was reported by Adams, Pérez de la Rosa and Charzaro (1990).
However, these reports presented the data on a TIC (total ion count) basis, but, currently, the use of FID (flame ionization detector) basis is preferred as being more accurate.

The purpose of this paper is to report on a new analysis of the leaf oils of *J. flaccida, J. martinezii and J. poblana* using FID quantitation plus a library of over 2,205 compounds (Adams 2007) for identification.

MATERIALS AND METHODS

Specimens collected: *J. flaccida* var. *flaccida*, *Adams 6892-6896*, 23 km e of San Roberto Junction on Mex. 60, Nuevo Leon, Mexico; *J. martinezii*, *Adams 5950-5952, 8709*, 40 km n of Lago de Moreno on Mex. 85 to Amarillo, thence 10 km e to La Quebrada Ranch, 21° 33.08' N, 101° 32.57' W, Jalisco, Mexico; *J. flaccida* var. *poblana*, *Zanoni 2637-2643*, 0.74 mi N of Amozo on old Rt. 150, Puebla, MX; *Adams 6868-6870*, 62 km s of Oaxaca, Mexico on Mex. 190. Voucher specimens are deposited at BAYLU.

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the

samples stored at 20 ºC until analyzed. The extracted leaves were oven dried (100 ºC, 48 h) for determination of oil yields.

Oils from 4-5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

Overall, the leaf oils of *J. flaccida* and *J. poblana* are similar and the oil of *J. martinezii* is quite different. The leaf oil of *J. flaccida* is dominated by α-pinene (65.0%) with moderate amounts of β-pinene (4.8%), myrcene (4.3%), limonene (3.5%), β-phellandrene (3.4%), linalool (2.9%) and manool oxide (3.5%). The oil of *J. poblana* is somewhat similar as it is dominated by α-pinene (52.9%) with moderate amounts of β-pinene (4.2%), myrcene (4.3%), limonene (2.2%), β-phellandrene (3.5%) and linalool (1.9%), but contains several unique compounds: δ-2-carene (1.8%), δ-3-carene (1.4%), trans-verbenol (2.7%), methyl chavicol (0.7%), and (E)-nerolidol (2.5%). The oil of *J. martinezii* was quite distinct with major components being α-pinene (16.6%), sabinene (10.4%) and camphor (11.1%) and moderate amounts of β-pinene (1.4%), myrcene (3.6%), limonene (1.8%), β-phellandrene (5.3%),linalool (2.8%), γ-terpinene (1.8%) and terpinen-4-ol (6.1%). It also contain several unique compounds: p-cymenene (0.7%), karahanaenone (1.3%), trans-dehydrocarvone (0.6%), trans-chrysanthenyl acetate (0.5%), linalool acetate (0.4%), noe-iso-3-thyjanyl acetate (0.8%), an aromatic phenol (KI 1320, 0.5%), trans-muurola-4(14), 5-diene (0.7%), epi-cubebol (0.5%), cubebol (1.1%), 1-epi-cubebol (1.0%), and an unknown diterpene (KI 1978, 0.6%).

ACKNOWLEDGEMENTS

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Table 1. Leaf essential oil compositions for *J. flaccida* (*Adams 6892*), *J. martinezii* (*Adams 5974*), and *J. poblana* (*Adams 2578*), based on FID gas chromatography. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.
* = Tentatively identified.

KI	Compound	*flaccida*	*poblana*	*martinezii*
921	**tricyclene**	**0.2**	**t**	**0.6**
924	**α-thujene**	**t**	**t**	**0.6**
932	**α-pinene**	**65.0**	**52.9**	**16.6**
945	α-fenchene	t	0.1	-
946	camphene	0.6	0.7	0.7
953	thuja-2,4-diene	t	0.2	0.1
961	verbenene	1.3	0.6	0.2
969	**sabinene**	**0.2**	**0.2**	**10.4**
974	1-octen-3-ol	-	-	-
974	**β-pinene**	**4.8**	**4.2**	**1.4**
988	myrcene	4.3	4.3	3.6
1001	**δ-2-carene**	**-**	**1.8**	**-**
1001	4-methyl, me-pentanoate*	0.1	-	-
1002	α-phellandrene	0.1	0.1	1.0
1008	**δ-3-carene**	**-**	**1.4**	**-**
1014	α-terpinene	t	t	1.0
1020	p-cymene	0.1	0.2	1.8
1024	limonene	3.5	2.2	1.8
1025	β-phellandrene	3.4	3.5	5.3
1032	(Z)-β-ocimene	t	t	t
1044	(E)-β-ocimene	1.5	0.7	0.4
1054	**γ-terpinene**	**0.2**	**0.1**	**1.8**
1065	cis-sabinene hydrate	-	-	0.6
1067	cis-linalool oxide (furanoid)	0.1	t	-
1086	terpinolene	0.5	0.7	0.8
1089	**p-cymenene**	**-**	**-**	**0.7**
1092	96, 109,43,152, C10-OH	1.0	0.3	1.8
1095	**linalool**	**2.9**	**1.6**	**2.8**
1112	3-m-3-buten-me-butanoate	0.2	-	-
1114	endo-fenchol	-	0.3	-
1118	cis-p-menth-2-en-1-ol	0.1	0.2	0.5
1122	α-campholenal	0.3	1.2	0.4
1133	cis-p-mentha-2,8-dien-1-ol	-	t	-
1135	trans-pinocarveol	0.3	1.1	0.8
1136	trans-p-menth-2-en-1-ol	-	-	-
1141	**camphor**	**0.5**	**0.6**	**11.1**
1141	**trans-verbenol**	**-**	**2.7**	**-**
1145	camphene hydrate	0.4	0.5	1.3
1148	citronellal	0.2	t	-
1154	**karahanaenone**	**-**	**-**	**1.3**
1155	iso-isopulegol	0.1	-	-
1160	**p-mentha-1,5-dien-8-ol**	**-**	**0.5**	**1.0**
1165	**borneol**	**0.7**	**0.6**	**-**
1172	cis-pinocamphone	0.2	0.3	0.3
1174	**terpinen-4-ol**	**0.3**	**0.3**	**6.1**
1178	**naphthalene**	**-**	**t**	**t**
1179	**p-cymen-8-ol**	**t**	**t**	**0.5**
1186	α-terpineol	0.4	0.7	0.7
1195	myrtenol	0.1	0.2	t
1195	myrtenal	-	-	0.1

KI	Compound	*flaccida*	*poblana*	*martinezii*
1195	**methyl chavicol**	**-**	**0.7**	**-**
1200	trans-dehydrocarvone	-	-	0.6
1204	**verbenone**	**t**	**0.6**	**0.5**
1215	**trans-carveol**	**0.1**	**0.7**	**-**
1218	endo-fenchyl acetate	-	-	-
1223	citronellol	0.1	-	-
1232	thymol, methyl ether	-	-	-
1235	**trans-chrysanthenyl acetate**	**-**	**-**	**0.5**
1239	carvone	-	0.2	-
1249	**piperitone**	**0.2**	**0.9**	**0.9**
1254	**linalool acetate**	**-**	**-**	**0.4**
1255	4Z-decenol	0.2	-	-
1284	bornyl acetate	0.4	1.1	1.8
1289	trans-sabinyl acetate	-	-	0.1
1289	**neo-iso-3-thyjanly acetate**	**-**	**-**	**0.8**
1289	thymol	-	0.2	-
1292	(2E,4Z)-decadienal	0.1	-	-
1315	(2E,4E)-decadienal	0.1	-	-
1320	**aromatic phenol 149,91,77,164**	**-**	**-**	**0.6**
1344	myrtenyl acetate	-	0.1	-
1345	α-terpinyl acetate	-	-	0.2
1345	α-cubebene	0.1	0.1	0.3
1396	duvalene acetate	-	-	-
1403	methyl eugenol	0.1	-	-
1417	**(E)-caryophyllene**	**0.2**	**0.3**	**0.1**
1448	cis-muurola-3,5-diene	-	-	-
1451	trans-muurola-3,5-diene	-	-	0.2
1452	α-humulene	-	t	-
1475	trans-cadina-1(6),4-diene	-	-	0.3
1484	**germacrene D**	**0.1**	**0.3**	**-**
1493	**trans-muurola-4(14),5-diene**	**-**	**-**	**0.7**
1493	**epi-cubebol**	**-**	**-**	**0.5**
1500	α-muurolene	-	t	-
1513	γ-cadinene	-	-	-
1514	**cubebol**	**-**	**-**	**1.1**
1521	trans-calamenene	-	t	0.5
1522	δ-cadinene	-	t	0.4
1528	zonarene	-	-	0.1
1533	trans-cadina-1,4-diene	-	-	t
1548	elemol	0.1	0.2	1.0
1555	elemicin	-	0.2	-
1561	**(E)-nerolidol**	**-**	**2.5**	**-**
1582	caryophyllene oxide	0.2	0.6	0.3
1627	**1-epi-cubenol**	**-**	**-**	**1.0**
1630	γ-eudesmol	-	-	t
1638	epi-α-cadinol	-	0.1	-
1638	epi-α-muurolol	-	0.1	-
1649	β-eudesmol	-	t	0.3
1652	α-eudesmol	-	0.1	0.3
1652	α-cadinol	-	0.1	-
1685	germacra-4(15),5,10-triene-1-al	-	-	-
1759	benzyl benzoate	-	-	-
1933	cyclohexadecanolide	-	-	-
1958	**iso-pimara-8(14),15-diene**	**0.1**	**-**	**1.0**
1978	**diterpene,43,81,147,243**	**-**	**-**	**0.6**
1987	manoyl oxide	3.0	0.3	1.0
2055	abietatriene	0.3	0.2	0.8

KI	Compound	*flaccida*	*poblana*	*martinezii*
2087	**abietadiene**	**-**	**-**	**2.3**
2056	manool	-	-	-
2105	iso-abienol	-	0.1	-
2264	**diterpene,43,55,271,286**	**-**	**t**	**-**
2331	trans-ferruginol	-	t	-

A new, flaccid, decurrent leaf variety of *Juniperus poblana* from Mexico: *J. poblana* var. *decurrens* R. P. Adams

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA Robert_Adams@baylor.edu

and

Andrea E. Schwarzbach
Department of Biomedicine, University of Texas at Brownsville, Brownsville, TX 78520, USA.

ABSTRACT

Analyses of nrDNA and four cp DNAs (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF) plus morphology and leaf essential oils revealed that the weeping (flaccid), decurrent leafed junipers near Topia, Durango are closely related to *J. poblana* (formerly *J. flaccida* var. *poblana*) and should be recognized as a new variety, *J. poblana* var. *decurrens* R. P. Adams **var. nov.** The leaf oil of *J. p.* var. *decurrens* is dominated by α-pinene (53.2%) with moderate amounts of β-pinene (5.3%), myrcene (4.3%), δ-2-carene (1.2%), δ-3-carene (2.5%), limonene (3.2%), β-phellandrene (3.1%), terpinolene (1.0%), (E)-caryphyllene (1.1%), and germacrene D (1.5%) and shares eleven unique terpenes with *J. poblana*. Published on-line www.phytologia.org *Phytologia 97(3): 152-163 (July 1, 2015).*

KEY WORDS: *Juniperus flaccida, J. martinezii, J. poblana, J. poblana* var. *decurrens* **var. nov.,** *Cupressaceae*, terpenes, leaf essential oil, morphology.

The flaccid leafed *Juniperus* of Mexico consist of three species: *J. flaccida* Schlecht. with large (9-12 mm diam.), multi-seeded [(4-)-6-10-(13)] cones; *J. poblana* (Martínez) R. P. Adams (formerly *J. flaccida* var. *poblana* Martínez) with very large (9-15 mm diam.), multi-seeded [(4-)-6-10-(13)] cones and *J. martinezii* Pérez de la Rosa with small seed cones (5-7 mm), 1-2 seeds per cone and foliage somewhat drooping but branchlets tips erect (Adams, 2014, Pérez de la Rosa. 1985). *Juniperus martinezii* is quite distinct in its morphology, but the other two taxa differ little in morphology with *J. flaccida* having radial branching and seed cones tan to brownish purple, whereas *J. poblana* has distichous foliage in vertical planes like *Thuja*, and not very flaccid (Zanoni and Adams, 1976, 1979; Adams, 2014) with bluish-brown seed cones. Each of these taxa has leaf margins that are hyaline and nearly entire, with either a few small teeth or merely a wavy margin (Adams, 2014). However, their DNA clearly places them in the serrate leaf margined *Juniperus* species of the western hemisphere with teethed margins secondarily lost (Adams, 2014).

Juniperus flaccida, J. martinezii and *J. poblana* have been treated as varieties of *J. flaccida*, until DNA sequencing of nrDNA (ITS) and trnC-trnD (Adams et al., 2006) revealed that *J. flaccida* varieties are not monophyletic and they recognized *J. f.* var. *martinezii* as *J. martinezii* and *J. f.* var. *poblana* as *J. poblana*. More recently, Adams and Schwarzbach (2013) published a detailed phylogeny of the serrate junipers of the western hemisphere based on nrDNA and four cp genes. They found *J. flaccida* (var. *flaccida*) in a group with *J. standleyi* (Fig. 1) and *J. poblana* (*J. f.* var. *poblana*) in a well supported sister group relationship. Likewise, *Juniperus martinezii* (*J. f.* var. *martinezii)* grouped with *J. durangensis* (Fig. 1) supported by high branch support. Their work appears to solidify support for the recognition of *J. martinezii* and *J. poblana.*

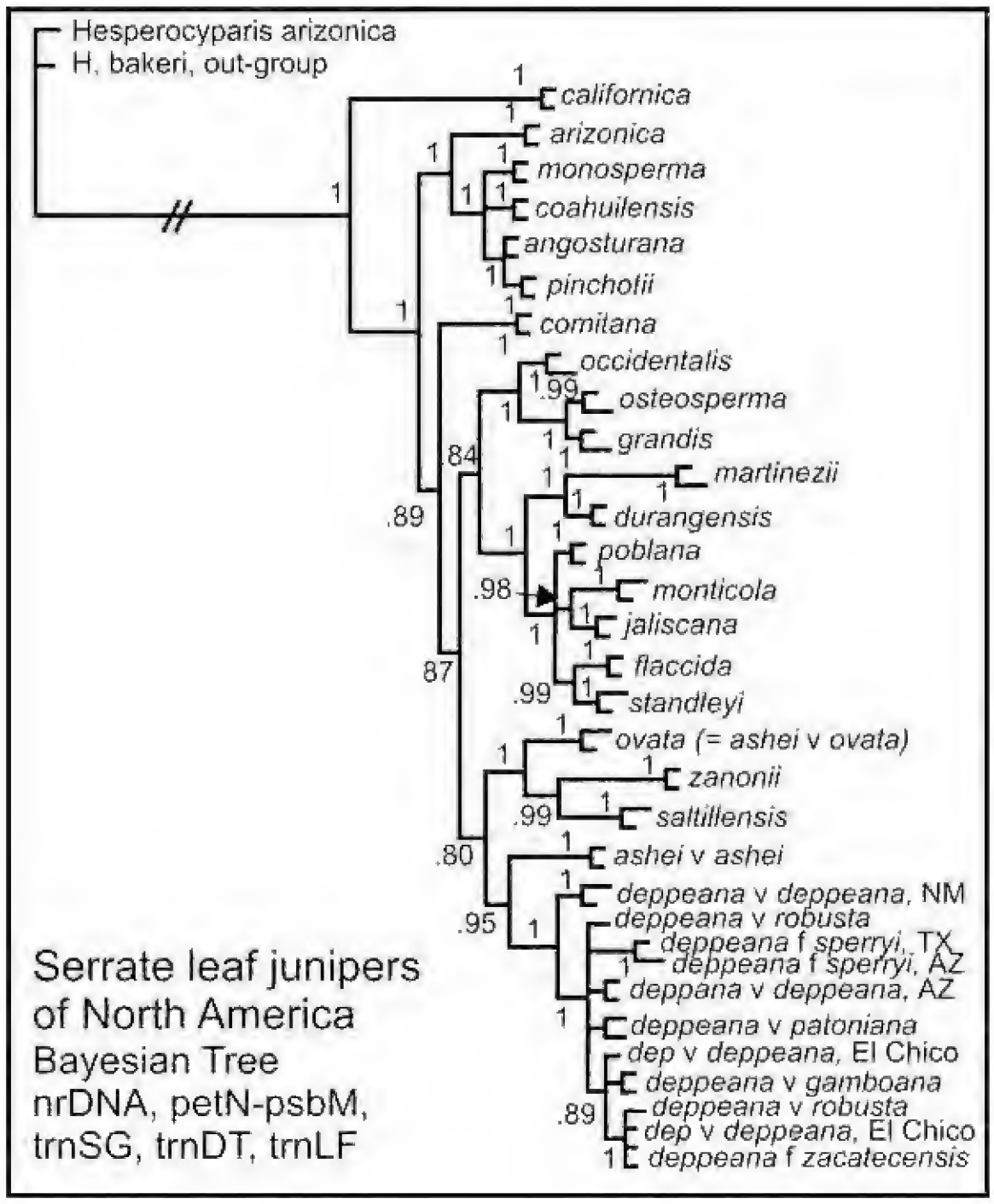

Figure 1. Bayesian analysis of the serrate leaf Juniperus of North America.
Numbers are posterior probabilities.
From Adams and Schwarzbach (2013). See text for discussion.

The differences in morphology and oil composition warrant the recognition of the decurrent leafed, flaccid foliaged, *Juniperus* as a new variety:

Juniperus poblana var. ***decurrens*** R. P Adams , **var. nov.** TYPE: Mexico, Durango, 2 km s of Valle de Topia, 25° 14' 11" N; 106° 26' 55.7" W, 1818 m, *R. P. Adams 11926*, 30 Jun 2009 (HOLOTYPE: BAYLU), Fig. 2.

Similar to *Juniperus poblana* and *J. flaccida*, but differing in having only decurrent leaves with free, divergent leaf tips.

Juniperus poblana var. *decurrens* is currently known only from the type locality where it is common on hillsides around Topia at about 1550-2000 m.

Other specimens studied: TOPOTYPES: *Adams 11927, 11928* (BAYLU); *S. González, M. González, I. L. López e Ing. José Soto 7269a, b* (BAYLU, CIIDIR, MEXU); Los Pinos, Valle de Topia, *A. García 1336* (CIIDIR, MEXU).

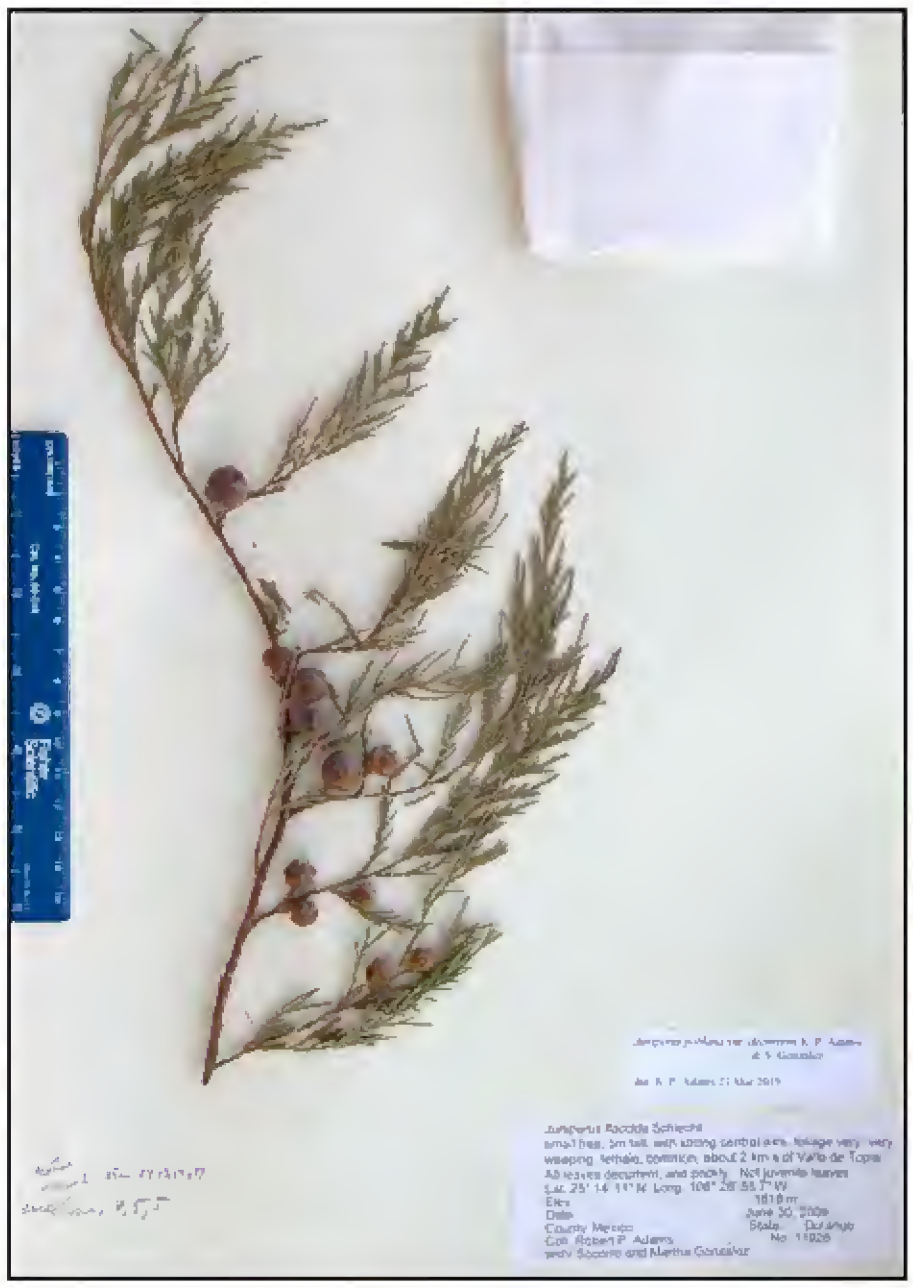

Fig. 2. Holotype of *J. poblana* var. *decurrens*.

Fig. 3. Leaves and seed cones of *J. poblana* var. *decurrens*.

Fig. 4. Habit of *J. poblana* var. *decurrens*.

Fig. 5. Leaf foliage, weeping.

Figure 6. Bark exfoliating in thin, scaly plates on *J. poblana* var. *decurrens*.

General description:
Dioecious. **Trees** to 10 m, branched with round crowns. **Trunk bark** brown exfoliating in thin, scaly plates. **Branches** very flaccid branchlets. **Leaves** all decurrent, with sharp, mucronate, usually divergent tips. **Seed cones** spherical, glaucous, bluish brown, 12-17 mm, mature cones usually show suture lines from fusion of cone-scales, appearing like a soccer ball. **Seeds** (4-)5-7(-9) per cone. **Pollen shed** spring. **Habitat** usually on dry slopes, in pure stands or in mixed forests, at 1550-2000 m elevation. **Uses** none known. **Dist**.: known only from type locality, Topia, Durango, Mexico. **Status**: limited distribution in areas that may be cleared for ranching, so it may become threatened in the future.

The purpose of the present paper is to compare the DNA sequences, volatile oils and morphology of *J. flaccida, J. martinezii*, *J. poblana* var. *poblana*, and *J. p.* var. *decurrens*.

The composition of the volatile leaf oils of *J. flaccida* and *J. poblana* (as *J. f.* var. *poblana*) were first reported by Adams, Zanoni and Hogge (1984). The composition of the leaf oil of *J. martinezii* was reported by Adams, Pérez de la Rosa and Cházaro (1990). Recently, Adams and Zanoni (2015) have reported on a re-examination the leaf oils of *J. flaccida, J. martinezii and J. poblana* using modern TIC-GC quantitation methods.

MATERIALS AND METHODS

Specimens collected: *Juniperus poblana* var. *decurrens, R. P. Adams 11926, 11927, 11928,* small trees, to 5 m tall, with strong central axis, foliage very, very, weeping, common, about 2 km s of Valle de Topia. All leaves decurrent, and prickly. Not merely juvenile leaves. 25° 14' 11" N; 106° 26' 55.7" W, 1818 m, 30 Jun 2009, Durango, Mexico; *J. flaccida* var. *flaccida*, *Adams 6892-6896*, 23 km e of San Roberto Junction on Mex. 60, Nuevo Leon, Mexico; *J. martinezii*, *Adams 5950-5952, 8709*, 40 km n of Lagos de Moreno on Mex. 85 to Amarillo, thence 10 km e to La Quebrada Ranch, 21° 33.08' N, 101° 32.57' W, Jalisco, Mexico; *J. poblana*, *Zanoni 2637-2643*, 0.74 mi N of Amozoc on old Rt. 150, Puebla, MX; *Adams 6868-6870*, 62 km s of Oaxaca, Mexico on Mex. 190.
Voucher specimens are deposited at BAYLU.

Fresh, air dried leaves (50-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at 20 ºC until analyzed. The extracted leaves were oven dried (100 ºC, 48 h) for determination of oil yields.

Oils from 4-5 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP 5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

DNA Amplifications and purification: see Adams, Bartel and Price (2009) and Adams and Kauffmann (2010). Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters. Available from http://www.geneious.com/) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v. 3.1 (Ronquist and Huelsenbeck, 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data, using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

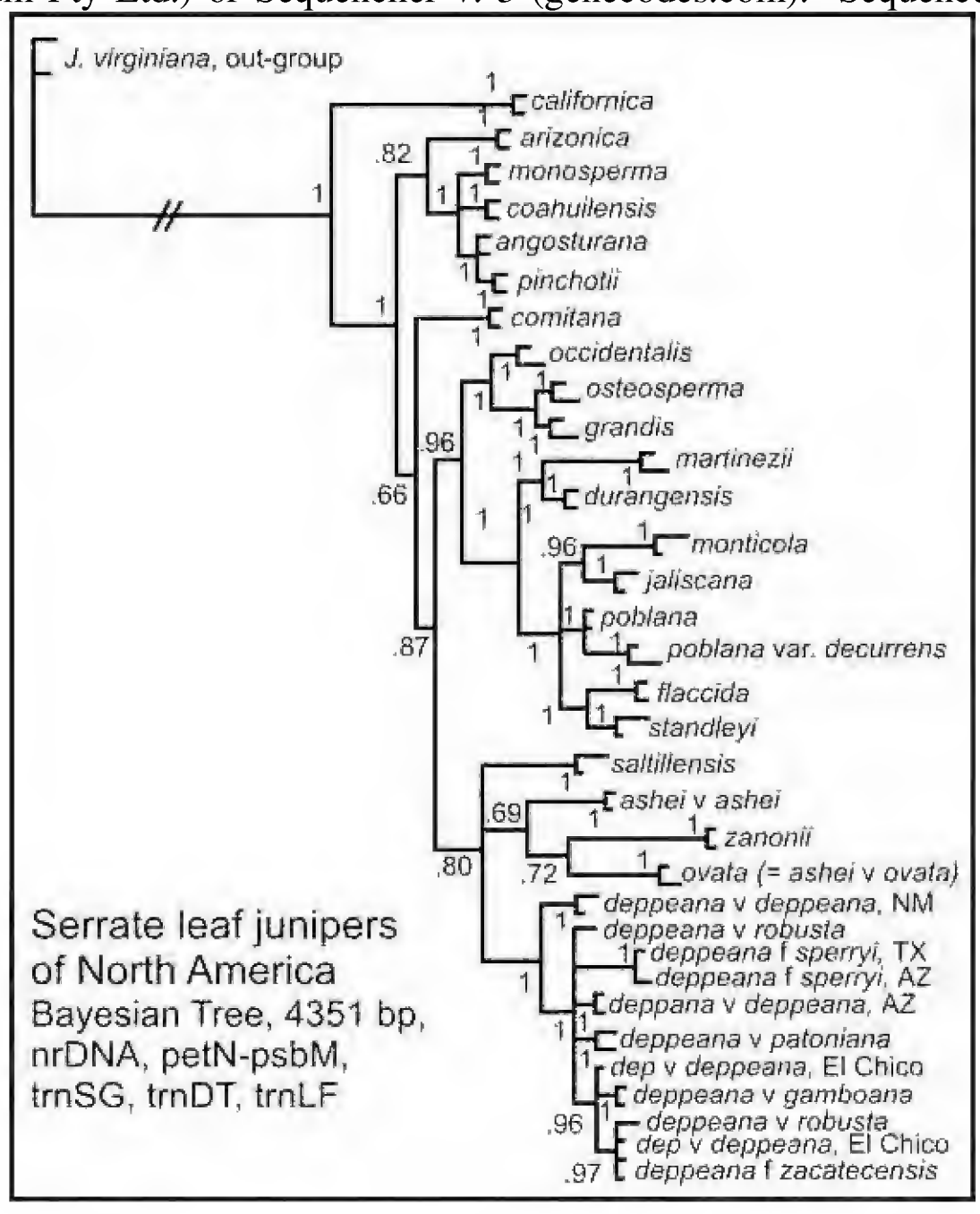

Fig. 7. Bayesian tree. Numbers next to the branch points are Posterior probabilities (0-1 scale).

RESULTS AND DISCUSSION

Sequencing nrDNA, petN-psbM, trnS-trnG, trnD-trnT, and trnL-trnF resulted in 4351 bp of data. Adding these data from *J. poblana* var. *decurrens* to other serrate leaf junipers of North America, gave 62 OTUs for Bayesian analysis. This analysis revealed that *J. p.* var. *decurrens* is in a clade with *J. poblana* (Fig. 7), and thence in a clade with a morphologically diverse group of junipers (*J. flaccida, J. standleyi, J. monticola* and *J. jaliscana*).

To examine the magnitude of the DNA differences, a minimum spanning network was constructed based on differences in mutational events (MEs = SNPs + indels). Only 4 MEs separate *J. poblana* and *J. p.* var. *decurrens* (Fig. 8). These 4 MEs consist of 2 SNPs in the nrDNA, and 1 SNP and 1 indel (1bp) in cp DNA. Notice that *J. flaccida* and *J. p.* var. *decurrens* are separated by 10 MEs (4 SNPs + 1 indel in nrDNA and 1 SNP + 4 indels in cpDNA). *Juniperus poblana* occupies a central node among a number of morphologically diverse species (Fig. 8).

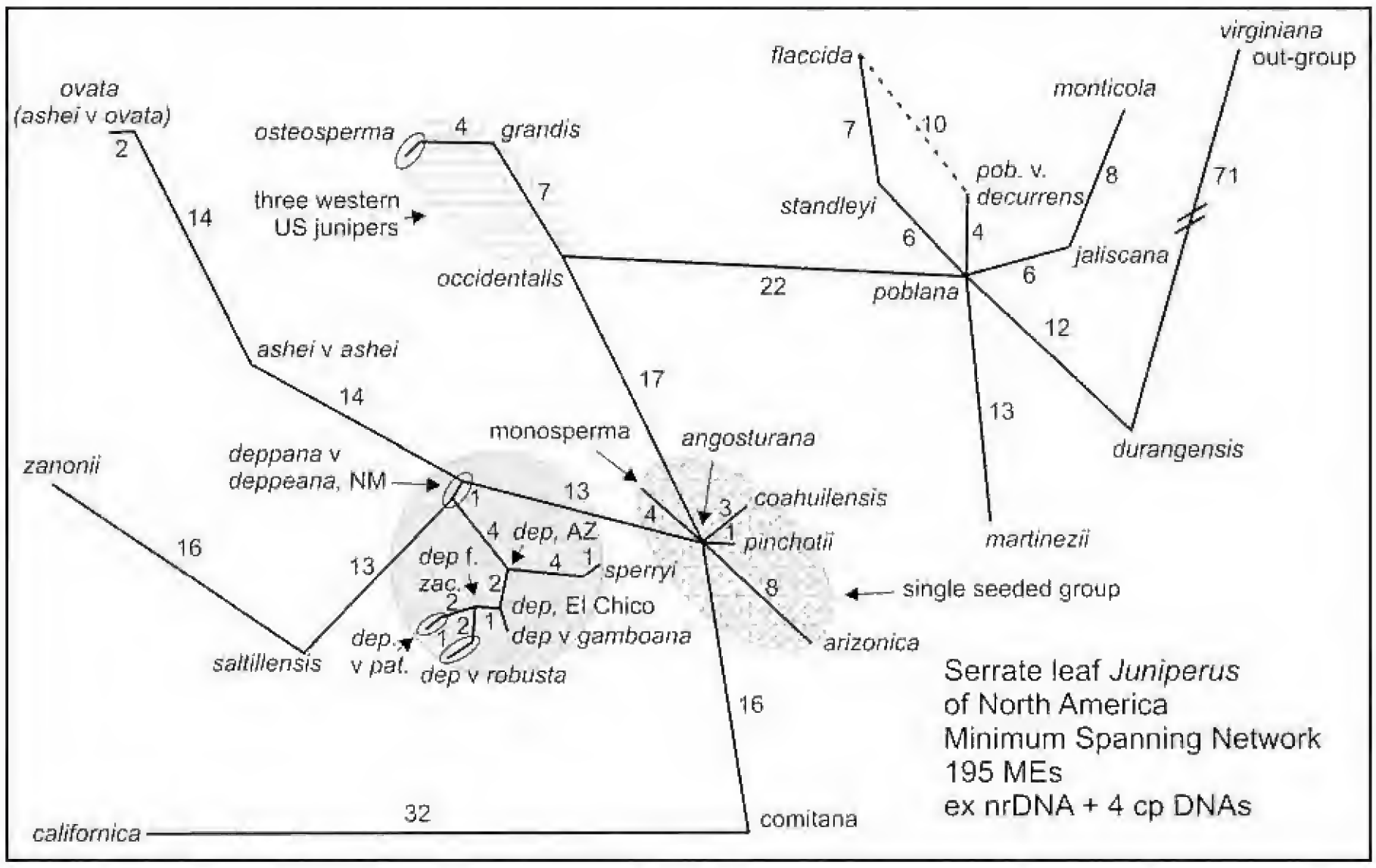

Figure 8. Minimum spanning network based on 195 MEs (SNPs + indels). The numbers next to the links are the number of MEs. The dashed line is the link between *J. flaccida* and *J. p.* var. *decurrens* (10 MEs) and is not a minimum link.

Analysis of the volatile leaf oils of *J. p.* var. *decurrens*, *J. poblana*, *J. flaccida* and *J. martinezii* is given in Table 1. Overall, the leaf oils of *J. flaccida* and *J. poblana* are similar and the oil of *J. martinezii* is quite different. The leaf oil of *J. p.* var. *decurrens* is dominated by α-pinene (53.2%) with moderate amounts of β-pinene (5.3%), myrcene (4.3%), δ-2-carene (1.2%), δ-3-carene (2.5%), limonene (3.2%), β-phellandrene (3.1%). terpinolene (1.0%), (E)-caryphyllene (1.1%), and germacrene D (1.5%). The leaf oil of *J. flaccida* is dominated by α-pinene (65.0%) with moderate amounts of β-pinene (4.8%), myrcene (4.3%), limonene (3.5%), β-phellandrene (3.4%), linalool (2.9%) and manool oxide (3.5%). The oil of *J. poblana* is somewhat similar as it is dominated by α-pinene (52.9%) with moderate amounts of β-pinene (4.2%), myrcene (4.3%), limonene (2.2%), β-phellandrene (3.5%) and linalool (1.6%). It contains only one unique compound: trans-verbenol (2.7%). The oil of *J. martinezii* was quite distinct with major components being α-pinene (16.6%), sabinene (10.4%) and camphor (11.1%) and moderate amounts of β-pinene (1.4%), myrcene (3.6%), limonene (1.8%), β-phellandrene (5.3%), linalool (2.8%), γ-terpinene (1.8%) and terpinen-4-ol (6.1%). It also contain several unique compounds: p-cymenene (0.7%), karahanaenone (1.3%), trans-dehydrocarvone (0.6%), trans-chrysanthenyl acetate (0.5%), linalool acetate (0.4%), noe0iso-3-thyjanyl acetate (0.8%), an aromatic phenol (KI 1320, 0.5%), trans-muurola-4(14), 5-diene (0.7%), epi-cubebol (0.5%), cubebol (1.1%), 1-epi-cubebol (1.0%), and an unknown diterpene (KI 1978, 0.6%).

It is interesting that *J. poblana* and *J. p.* var. *decurrens* have similar amounts of α-pinene (52.6, 53.2%) and share eleven unique components: δ-2-carene, δ-3-carene, endo-fenchol, methyl chavicol, elemicin, (E)-nerolidol, epi-α-eudesmol, epi-α-muurolol, α-cadinol, KI2264 (diterpene) and trans-ferruginol (Table 1).

The morphology of the leaves of *J. p.* var. *decurrens* is particular. There are three principal leaf types in *Juniperus* (Fig. 9): acicular (sections *Caryocedrus* and *Juniperus*); decurrent (section *Sabina*) and scale-like leaves (section *Sabina*). Within section *Sabina* there are several subtypes of leaves (Fig. 10).

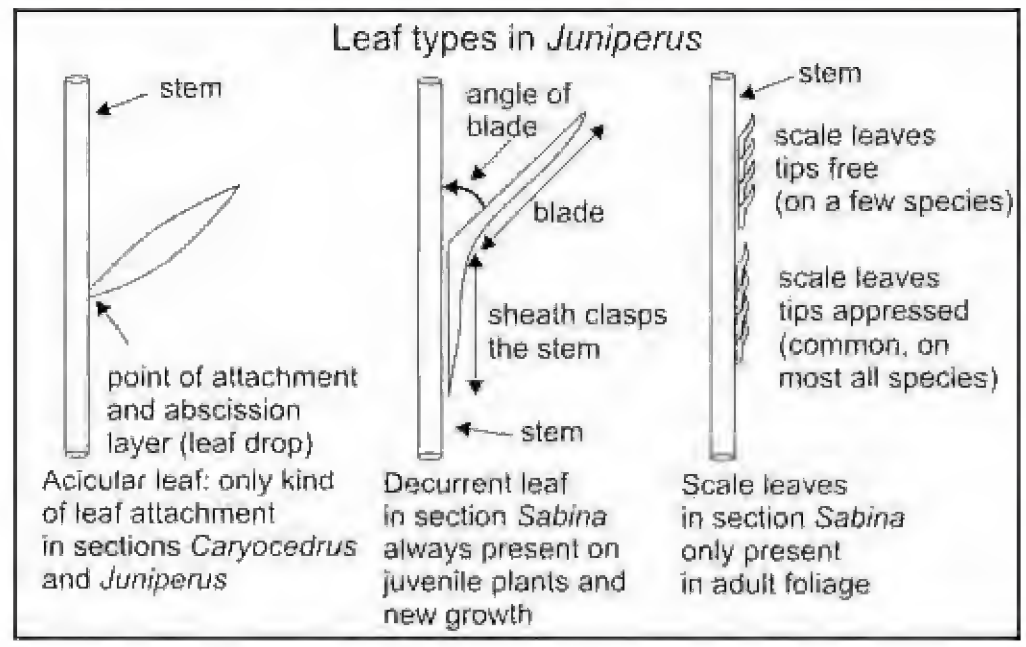

Figure 9. Three basic leaf types in *Juniperus*.

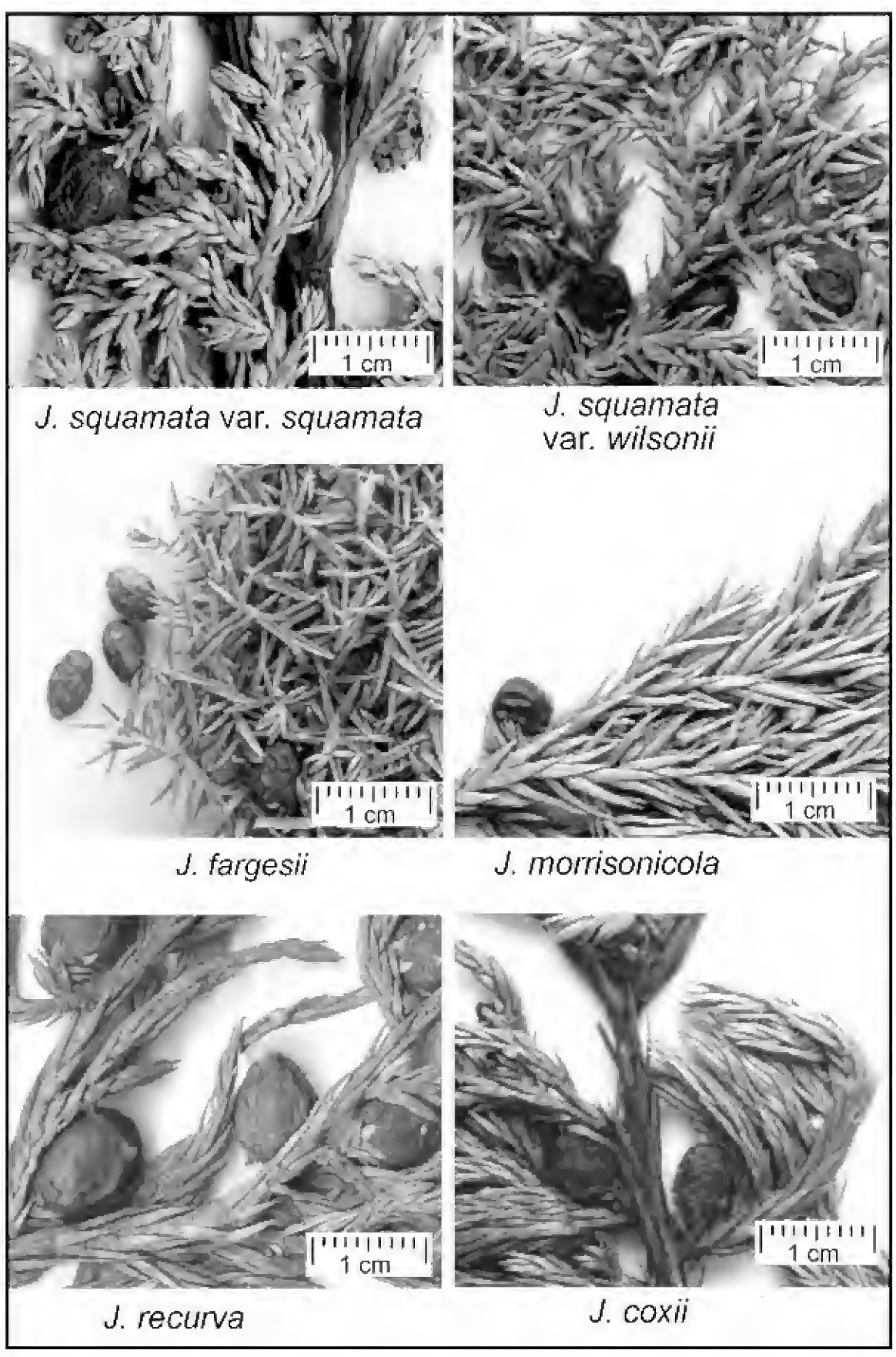

Figure 10. Variation in leaf types in section *Sabina* (left). Some of these types are confined to a species and some vary within and among species. None of these taxa have typical scale leaves (see *J. standleyi*, insert below). Note also decurrent leaves of *J. poblana* var. *decurrens* below.

Decurrent leaves of *J. poblana* var. *decurrens*

A comparison of the morphology of *J. poblana, J. p.* var. *decurrens* and *J. flaccida* is given in Table 2. The taxa are difficult to separate. A key to these taxa (plus *J. martinezii*) follows:

1. Seed cones large, 9-17 mm diam., (4-) 6-10 (-13) seeds per cone, terminal branch tips drooping (hanging)
    2. Foliage flaccid, but not weeping, branching planate, seed cones bluish-brown .....*J. poblana* var. *poblana*
    2. Foliage weeping, branching radially, seed cones brownish-purple, tan-brown
        3. All leaves decurrent, with free tips, foliage very weeping, bark exfoliating in thin, scaly, plates .....*J. poblana* var. *decurrens*
        3. Leaves decurrent with mostly appressed tips, foliage weeping, bark exfoliating in thick, interlaced stripes.....*J. flaccida*
1. Seed cones small, (5-) 6 (-9) mm diam., 1-2 (-3) seeds per cone, terminal branch tips erect .....*J. martinezii*

Table 2. Comparison of morphology of *J. poblana, J. p.* var. *decurrens* and *J. flaccida.*

	J. poblana var. *poblana*	*J. p.* var. *decurrens*	*J. flaccida*
leaves	decurrent leaves (DL) with free tips and some modified DL with appressed tips.	decurrent leaves (DL) with free tips and many modified DL with free tips.	decurrent leaves (DL) with free tips and many modified DL with appressed tips.
leaf tips	mucronate tips on DL	mucronate tips on DL and mod. DL	mucronate tips on DL, acute on modified DL
leaf gland	about 1/2 DL length	about 1/2 DL length about 1/2 mod. DL length	about 3/4 DL length about 1/2 mod. DL length
bark exfoliation pattern	thin, narrow, interlaced strips	thin, scaly plates	thick, interlaced strips
seed cones	9-15 mm, bluish brown	12-17 mm, bluish brown to purplish brown	9-12 mm, tan-brown to brownish purple
seeds per cone	(4-) 6-10 (-13)	(4-) 5-7 (-9)	(4-) 6-10 (-13)

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Table 1. Leaf essential oil compositions for *J. flaccida* (*Adams 6892*), *J. poblana* var. *decurrens* (*Adams 11932*), *J. poblana* var. *poblana* (*Adams 2578*), and *J. martinezii* (*Adams 5974*) based on FID gas chromatography and GCMS identification. Those compounds that appear to distinguish taxa are in boldface.

KI	Compound	*flaccida*	*decurrens*	*poblana*	*martinezii*
921	**tricyclene**	**0.2**	**t**	**t**	**0.6**
924	**α-thujene**	**t**	**t**	**t**	**0.6**
932	**α-pinene**	**65.0**	**53.2**	**52.9**	**16.6**
945	α-fenchene	t	0.1	0.1	-
946	camphene	0.6	0.5	0.7	0.7
953	thuja-2,4-diene	t	t	0.2	0.1
961	verbenene	1.3	0.1	0.6	0.2
969	**sabinene**	**0.2**	**-**	**0.2**	**10.4**
974	1-octen-3-ol	-	0.1	-	-
974	**β-pinene**	**4.8**	**5.3**	**4.2**	**1.4**
988	myrcene	4.3	5.6	4.3	3.6
1001	**δ-2-carene**	**-**	**1.2**	**1.8**	**-**
1001	4-methyl, me-pentanoate*	0.1	-	-	-
1002	α-phellandrene	0.1	0.1	0.1	1.0
1008	**δ-3-carene**	**-**	**2.5**	**1.4**	**-**
1014	α-terpinene	t	t	t	1.0
1020	p-cymene	0.1	t	0.2	1.8
1024	limonene	3.5	3.2	2.2	1.8
1025	β-phellandrene	3.4	3.1	3.5	5.3
1032	(Z)-β-ocimene	t	0.1	t	t
1044	(E)-β-ocimene	1.5	1.8	0.7	0.4
1054	**γ-terpinene**	**0.2**	**0.1**	**0.1**	**1.8**
1065	cis-sabinene hydrate	-	-	-	0.6
1067	cis-linalool oxide (furanoid)	0.1	-	t	-
1086	terpinolene	0.5	1.0	0.7	0.8
1089	**p-cymenene**	**-**	**-**	**-**	**0.7**
1092	96, 109,43,152, C10-OH	1.0	-	0.3	1.8
1095	**linalool**	**2.9**	**0.7**	**1.6**	**2.8**
1112	3-m-3-buten-me-butanoate	0.2	-	-	-
1114	endo-fenchol	-	0.1	0.3	-
1118	cis-p-menth-2-en-1-ol	0.1	0.1	0.2	0.5
1122	α-campholenal	0.3	0.1	1.2	0.4
1133	cis-p-mentha-2,8-dien-1-ol	-	-	t	-
1135	trans-pinocarveol	0.3	-	1.1	0.8
1136	trans-p-menth-2-en-1-ol	-	0.2	-	-
1141	**camphor**	**0.5**	**0.3**	**0.6**	**11.1**
1141	**trans-verbenol**	**-**	**-**	**2.7**	**-**
1145	camphene hydrate	0.4	0.2	0.5	1.3
1148	citronellal	0.2	-	t	-
1154	**karahanaenone**	**-**	**-**	**-**	**1.3**
1155	iso-isopulegol	0.1	-	-	-
1160	**p-mentha-1,5-dien-8-ol**	**-**	**-**	**0.5**	**1.0**
1165	**borneol**	**0.7**	**0.7**	**0.6**	**-**
1172	cis-pinocamphone	0.2	0.1	0.3	0.3
1174	**terpinen-4-ol**	**0.3**	**0.2**	**0.3**	**6.1**
1178	**naphthalene**	**-**	**0.4**	**t**	**t**
1179	**p-cymen-8-ol**	**t**	**t**	**t**	**0.5**
1186	α-terpineol	0.4	0.9	0.7	0.7
1195	myrtenol	0.1	-	0.2	t
KI	Compound	flac6892	fjuv1932	pob2578	mart5974
1195	myrtenal	-	-	-	0.1
1195	**methyl chavicol**	**-**	**0.8**	**0.7**	**-**

KI	Compound	*flaccida*	*decurrens*	*poblana*	*martinezii*
1200	trans-dehydrocarvone	-	-	-	0.6
1204	**verbenone**	**t**	**t**	**0.6**	**0.5**
1215	**trans-carveol**	**0.1**	**-**	**0.7**	**-**
1218	endo-fenchyl acetate	-	0.1	-	-
1223	citronellol	0.1	-	-	-
1232	thymol, methyl ether	-	0.1	-	-
1235	**trans-chrysanthenyl acetate**	**-**	**-**	**-**	**0.5**
1239	carvone	-	-	0.2	-
1249	**piperitone**	**0.2**	**0.1**	**0.9**	**0.9**
1254	**linalool acetate**	**-**	**-**	**-**	**0.4**
1255	4Z-decenol	0.2	-	-	-
1284	bornyl acetate	0.4	0.8	1.1	1.8
1289	trans-sabinyl acetate	-	-	-	0.1
1289	**neo-iso-3-thyjanly acetate**	**-**	**-**	**-**	**0.8**
1289	thymol	-	-	0.2	-
1292	(2E,4Z)-decadienal	0.1	-	-	-
1315	(2E,4E)-decadienal	0.1	-	-	-
1320	**aromatic phenol 149,91,77,164**	**-**	**-**	**-**	**0.6**
1344	myrtenyl acetate	-	-	0.1	-
1345	α-terpinyl acetate	-	-	-	0.2
1345	α-cubebene	0.1	-	0.1	0.3
1396	duvalene acetate	-	0.3	-	-
1403	methyl eugenol	0.1	0.3	-	-
1417	**(E)-caryophyllene**	**0.2**	**1.1**	**0.3**	**0.1**
1448	cis-muurola-3,5-diene	-	0.2	-	-
1451	trans-muurola-3,5-diene	-	-	-	0.2
1452	α-humulene	-	-	t	-
1475	trans-cadina-1(6),4-diene	-	0.1	-	0.3
1484	**germacrene D**	**0.1**	**1.5**	**0.3**	**-**
1493	**trans-muurola-4(14),5-diene**	**-**	**0.1**	**-**	**0.7**
1493	**epi-cubebol**	**-**	**-**	**-**	**0.5**
1500	α-muurolene	-	-	t	-
1513	γ-cadinene	-	0.2	-	-
1514	**cubebol**	**-**	**0.4**	**-**	**1.1**
1521	trans-calamenene	-	-	t	0.5
1522	δ-cadinene	-	0.4	t	0.4
1528	zonarene	-	0.1	-	0.1
1533	trans-cadina-1,4-diene	-	-	-	t
1548	elemol	0.1	-	0.2	1.0
1555	elemicin	-	0.4	0.2	-
1561	**(E)-nerolidol**	**-**	**0.9**	**2.5**	**-**
1582	caryophyllene oxide	0.2	0.8	0.6	0.3
1627	**1-epi-cubenol**	**-**	**0.7**	**-**	**1.0**
1630	γ-eudesmol	-	-	-	t
1638	epi-α-cadinol	-	0.8	0.1	-
1638	epi-α-muurolol	-	0.8	0.1	-
1649	β-eudesmol	-	-	t	0.3
1652	α-eudesmol	-	-	0.1	0.3
1652	α-cadinol	-	0.8	0.1	-
1685	germacra-4(15),5,10-triene-1-al	-	0.8	-	-
1759	benzyl benzoate	-	t	-	-
1933	cyclohexadecanolide	-	t	-	-
1958	**iso-pimara-8(14),15-diene**	**0.1**	**-**	**-**	**1.0**
1978	**diterpene,43,81,147,243**	**-**	**-**	**-**	**0.6**
1987	manoyl oxide	3.0	0.6	0.3	1.0
KI	Compound	flac6892	fjuv1932	pob2578	mart5974
2055	abietatriene	0.3	0.1	0.2	0.8

KI	Compound	*flaccida*	*decurrens*	*poblana*	*martinezii*
2087	**abietadiene**	**-**	**-**	**-**	**2.3**
2056	manool	-	0.1	-	-
2105	iso-abienol	-	-	0.1	-
2264	**diterpene,43,55,271,286**	**-**	**0.8**	**t**	**-**
2331	trans-ferruginol	-	0.2	t	-

KI = Kovats Index (linear) on DB-5 column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Preliminary study of variation in leaf volatile oil of *Borrichia frutescens* (L.) DC. along the Texas Gulf Coast

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
Robert_Adams@baylor.edu

Sean T. Carver and Michael A. Arnold
Department of Horticultural Sciences, TAMU 2133
Texas A & M University, College Station, TX 77843-2133

ABSTRACT

Borrichia frutescens from along the Texas Gulf Coast were grown in a common garden at College Station, TX. Analysis of the volatile leaf oils revealed a very unusual mosiac among individuals. The oils of individuals from South Padre Island grouped with individuals from near Galveston. No clear pattern of geographic variation was discerned. The volatile oil is often dominated by sabinene (16.2 - 30.35), but two individuals had 1.5 and 5.8%. β-phellandrene was the major component in several plants (17.6-21.0%). The major component for one plant was germacrene D (18.0%). Sixty-two terpenoids plus benzene aldehyde were identified in the oils.
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KEY WORDS: *Borrichia frutescens,* Asteraceae, terpenes, leaf essential oil, geographic variation.

Borrichia frutescens is a halophyte that grows on beaches, dunes, barrier islands in saline or brackish areas. It ranges along the Atlantic and Gulf Coast from Virginia to Florida and thence, westward to Texas and south to Yucatan as well as throughout the West Indies and Bermuda (Semple, 1978). Oddly, it ranges inland along the Rio Grande in south Texas and has a disjunct population in Coahuila (Semple, 1978).

Evaluation of plants from the Texas Gulf Coast for possible horticultural use in a common nursery at College Station, TX (30° 37' 24.24", -97° 22' 0.17") afforded an opportunity to examine the leaf volatile oils, free of environmental effects due to different habitats. Samples were obtained from plants grown in #3 black plastic containers filled with the commercial substrate Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, BC) amended with 6.53 kg·m-3 15N -3.9P-9.9K controlled release 3 to 4 month formulation fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio). Plants from which tissues were harvested were at a mature flowering growth stage, grown in full sun, manually weeded, and irrigated as needed to maintain leaf turgidity.

The phytochemistry of *B. frutescens* has been little studied. There is a report on triterpenoids with sitgmastanol, stigmasterol, oleanoic aicd and zoapatanolide A found in *B. frutescens* (Delgado et al., 1992). Cantrell et al. (1996) reported several new triterpenes in *B. frutescens.* There have, apparently, been no reports on the leaf volatile oil of *Borrichia*.

The purpose of the present work was to report on a comprehensive analysis of the steam volatile leaf oils of *B. frutescens* and give a preliminary analysis of variation among individuals.

MATERIALS AND METHODS

Specimens were collected (SC) and grown in a common nursery on the campus of Texas A & M, College Station, TX. Specimens used in this study, *Borrichia frutescens* :

S. Carver B3, *Adams lab 14235*, 26° 06.742' N, 97° 10.212' W, Laguna St. and Campeche, S. Padre Isl., TX; *S. Carver B4*, *Adams lab 14236*, 27° 17.363' N, 97° 39.710' W, End of Rd. 771, Rivera Beach, 9.4 mi. e of Rivera, TX; *Carver B6*, *Adams lab 14237,* 26° 08.435' N, 97° 10.492' W, Convention Center, South Padre Island, TX; *S. Carver B7*, *Adams lab 14238*, 26° 04.353' N, 97° 22.510' W, next to Whataburger, Port Isabel, TX; *S. Carver B8*, *Adams lab 14239*, 26° 04.715' N, 97° 12.712' W, Shore Dr. Port Isabel, TX; *S. Carver B10*, *Adams lab 14240*, 26° 07.175' N, 97° 09.945' W, Gulf and E. Mars streets, S. Padre Island, TX; *S. Carver B16*, *Adams lab 14241*, 28° 33.601' N, 96° 32.247' W, Public Beach, Magnolia Beach, TX; *S. Carver B21*, *Adams lab 14242*, 29° 22.040' N, 94° 45.607' W, Hwy 87, side of road, Port Bolivar, TX; *S. Carver B22*, *Adams lab 14243*, 29° 40.091' N, 94° 04.279' W, on beach, McFaddin NWR, 16 mi sw Port Arthur, TX; *S. Carver B23*, *Adams lab 14244*, 29° 42.612' N, 93° 51.539' W, on bank of Sabine River, 1st Ave., Sabine, TX; *S. Carver B25*, *Adams lab 14245*, 28° 56.415' N, 95° 17.888' W, on Bay Beach Park View, Surfside, TX; *S. Carver B26*, *Adams lab 14246,* 29° 33.079' N, 94° 22.336' W, in ditch, jct. TX 124 and 87, High Island, TX.

Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20ºC until analyzed. The extracted leaves were oven dried (100ºC, 48 h) for determination of oil yields. Oils were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

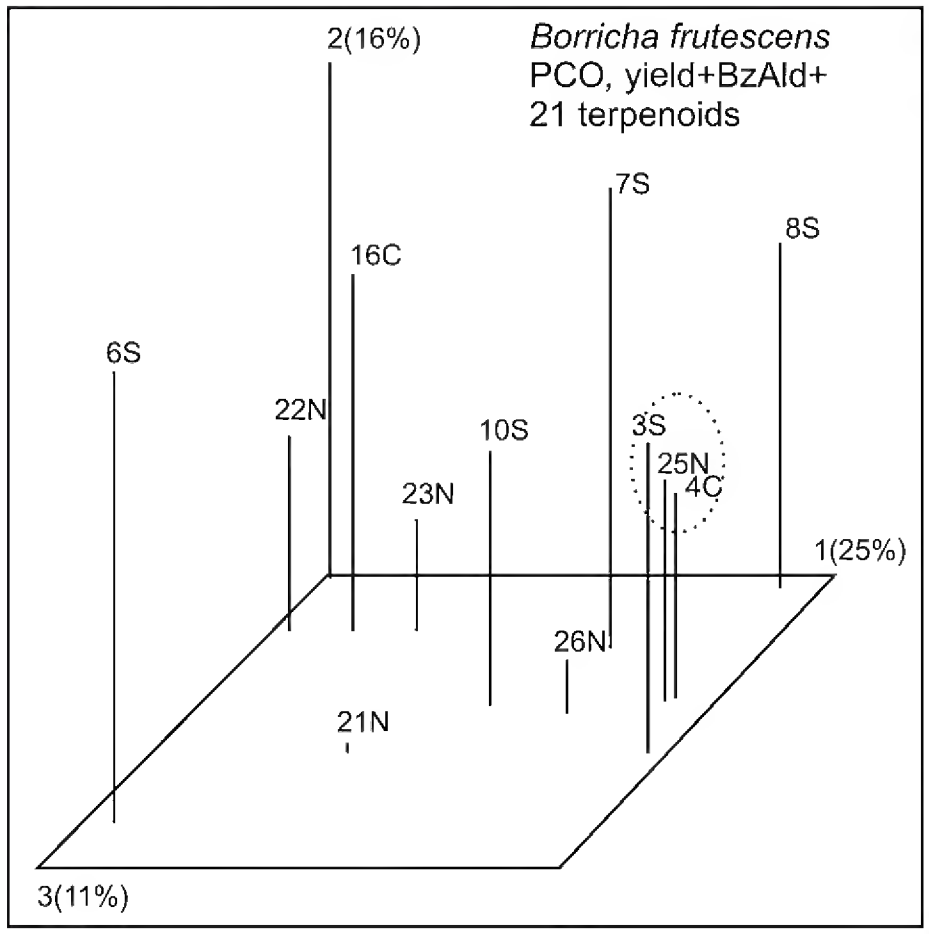
RESULTS AND DISCUSSION

The leaf oils of *Borrichia frutescens* were clear except B10, which was faint yellow (Table 1). The yields ranged from 0.29 to 0.70%, except for plant B6 that had a yield of 1.86%. Thus, there appears to be a polymorphism in oil production.

The composition of the volatile leaf oils revealed a very unusual mosiac among individuals (Table 2). The volatile leaf oil of *Borrichia frutescens* is often dominated by sabinene (16.2 - 30.35, plants B21, B23, B10, B6, Table 2), but two individuals had 1.5 and 5.8% (plants B4, B7, Table 2). β-phellandrene was the major component for B4 (21.0%) and B26 (20.3%). The major component for B7 was germacrene D (18.0%). Plants B6, 7 and 10 are from a small area on south Padre Island, yet their oils vary widely (Table 2). Note also the considerable variation among B21, 23, 26 from the northern region (Table 2). It might be noted that benzene aldehyde is one of the few non-terpenoids in the volatile leaf oil and it varies continuously from 2.3 to 5.4% (Table 2). Sixty-two terpenoids plus benzene aldehyde were identified in the oils.

To visualize the overall similarities among the volatile leaf oils, similarities were computed among individuals using 22 components plus % oil yield. Principal Coordinates Ordination (PCO) of the

matrix of similarities resulted in five eigenroots before they began to asymptote. These eigenroots accounted for 25.2, 16.5, 11.4, 9.7 and 7.9% of the variance among individuals (70.7% of total). The lack of loading on the first three eigenroots indicates that there are multiple modes of variation in oils among these individuals. PCO ordination shows (Fig. 1) this is indeed the case. Notice the lack of tight groups in the ordination (Fig. 1) with only plants 3S, 4C, 25N from south, central and north in a group. Plants 6S, 7S, 8S, all from the south Padre Island area, are quite distinct in their volatile oils.



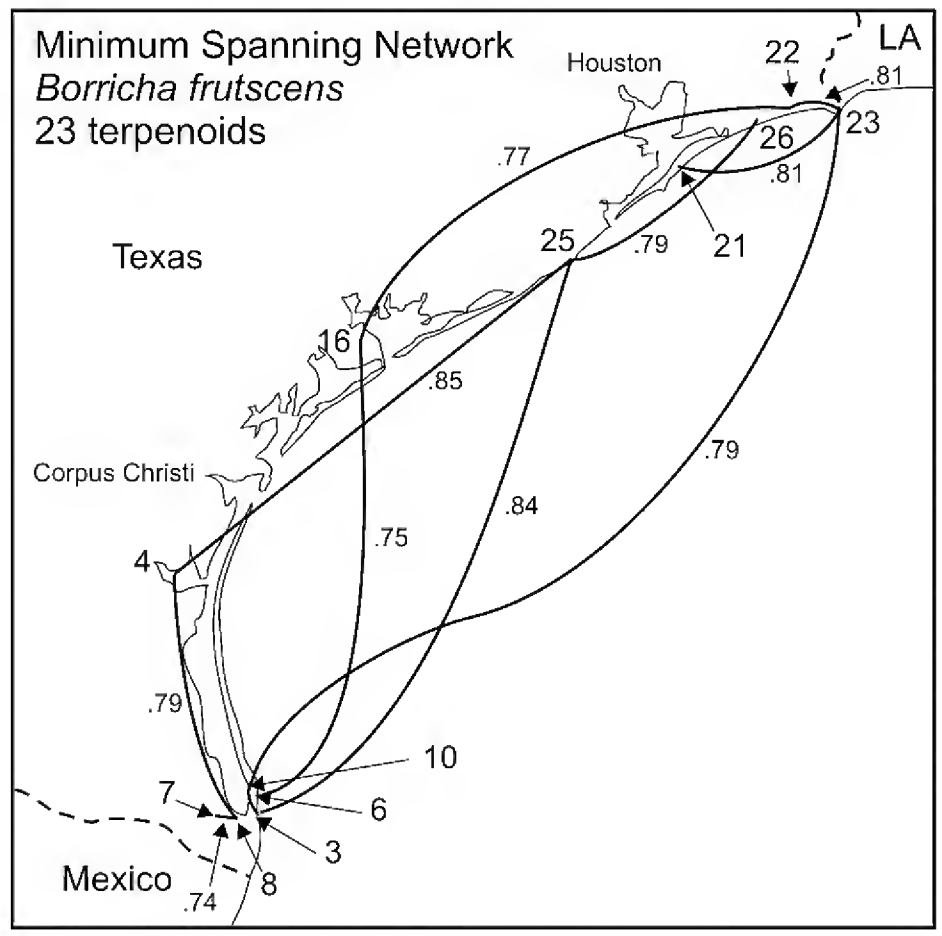


Figure 1. PCO of *Borrichia frutescens* individuals. Figure 2. Minimum spanning network.

In order to examine geographic variation, a minimum spanning network was constructed to link the nearest neighbor for each plant based on the 23 oil characters. Figure 2 shows that 4 of the plants from the south (south Padre Island area) have their most similar oil to that of a plant from the northern or central area (cf. 3-25, 6-16, 10-23, 8-4, Fig. 2). Only plant 7 is most similar to a nearby plant 8 (Fig. 2). A similar pattern is found among the northern plants. The clearest geographical pattern is a north-south network of similar oil patterns. Of course, this is only a preliminary study based on limited samples. The lack of clustering among the five southern plants from a small area is remarkable. However, it is interesting to note that Carver et (2015) reported that analysis of morphological data did not cluster neatly into regional groups.

ACKNOWLEDGEMENTS

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Table 1. Yields (percent oil on foliage oven dry wt. basis) of volatile leaf oils for *Borrichia frutescens* individuals.

plant	% yield	oil color
B3, 14235	0.64	clear
B4, 14236	0.70	clear
B6, 14237	1.86	clear
B7, 14238	0.52	clear
B8, 14239	0.43	clear
B10, 14240	0.60	faint yellow
B16, 14241	0.58	clear
B21, 14242	0.43	clear
B22, 14243	0.29	clear
B23, 14244	0.48	clear
B25, 14245	0.52	clear
B26, 14246	0.38	clear

Table 2. Leaf essential oil compositions for *Borrichia frutescens* individuals based on FID gas chromatography. KI = Kovats Index (linear) on DB-5 column. Those compounds that appear to distinguish individuals are in boldface. Components with an * were used in similarity calculations. Southern sites: B6-S, B7-S, B10-S; Central: B4-C; Northern: B21-N, B23-N, B26-N.

KI	Compound	B4-C	B26-N	B21-N	B23-N	B10-S	B6-S	B7-S
	% oil yields (w/DW)*	0.70	0.38	0.43	0.48	0.60	1.86	0.52
846	(2E)-hexenal	0.2	0.2	1.1	0.2	0.2	0.2	0.3
921	tricyclene	0.2	0.2	0.1	0.1	0.2	0.1	0.2
924	α-thujene	t	t	0.3	0.2	0.3	0.2	0.1
932	**α-pinene***	**8.3**	**8.3**	**4.4**	**3.8**	**6.2**	**4.4**	**6.7**
946	**camphene***	**10.4**	**9.1**	**4.2**	**3.5**	**7.3**	**5.2**	**8.6**
969	**sabinene***	**1.5**	**10.2**	**30.3**	**27.0**	**25.4**	**16.2**	**5.8**
974	**β-pinene***	**7.9**	**7.2**	**4.1**	**3.7**	**6.2**	**5.1**	**8.1**
988	**myrcene***	**0.6**	**7.8**	**3.4**	**2.6**	**1.2**	**3.7**	**1.1**
1003	**p-menthal-1(7),8-diene***	**t**	**3.2**	**2.9**	**1.9**	**t**	**t**	**t**
1014	α-terpinene	-	0.2	0.5	0.4	0.2	0.4	0.1
1020	p-cymene	-	t	0.3	t	t	t	t
1024	**limonene***	**10.7**	**10.2**	**9.0**	**6.5**	**6.9**	**5.1**	**4.7**
1025	**β-phellandrene***	**21.0**	**20.3**	**17.6**	**12.7**	**13.3**	**10.2**	**9.0**
1032	(Z)-β-ocimene	0.3	0.2	0.3	0.2	2.1	0.2	0.3
1036	**benzene aldehyde***	**4.3**	**2.3**	**4.8**	**3.1**	**3.3**	**3.2**	**5.4**
1054	γ-terpinene	t	0.4	1.6	0.8	0.5	0.6	0.3
1065	cis-sabinene hydrate	t	0.1	0.4	0.3	0.2	0.3	0.1
1085	p-mentha-2,4(8)-diene	t	0.1	0.2	0.2	0.1	0.2	0.1
1098	trans-sabinene hydrate	t	0.2	0.4	0.2	0.2	0.3	t
1100	n-nonanal	-	t	0.1	t	t	t	t
1118	cis-p-menth-2-en-1-ol	t	t	0.2	0.1	t	0.2	t
1136	trans-p-menth-2-en-1-ol	t	t	t	t	-	0.1	t
1141	**camphor***	**t**	**t**	**t**	**-**	**0.9**	**t**	**t**
1165	**borneol***	**0.7**	**1.7**	**0.2**	**0.3**	**0.3**	**t**	**2.3**
1174	**terpinen-4-ol***	**0.1**	**0.5**	**1.6**	**1.2**	**0.7**	**1.4**	**0.4**
1183	cryptone	t	0.2	0.2	t	-	t	t
1186	α-terpineol	t	t	t	t	-	t	t
1201	n-decanal	t	t	t	-	-	t	t
1215	2-methyl benzaldehyde	t	t	-	t	-	0.2	t
1284	**bornyl acetate***	**11.4**	**5.7**	**1.7**	**3.1**	**5.9**	**1.3**	**3.0**
1309	**p-vinyl guaiacol***	**0.3**	**0.1**	**0.6**	**0.1**	**t**	**0.7**	**t**
1335	δ-elemene	t	t	-	-	t	0.2	t
1374	α-copaene	t	t	-	t	t	t	t
1387	β-cubebene	0.6	0.1	t	0.6	0.4	t	0.4
1411	α-cis bergamotene	t	0.1	t	0.2	-	t	t
1417	(E)-caryophyllene	0.6	0.3	0.8	0.8	0.2	1.3	1.0
1430	β-copaene	t	t	-	t	-	-	t
1432	trans-α-bergamotene	0.6	0.4	0.5	0.5	t	t	0.3
1452	**α-humulene***	**0.3**	**0.1**	**0.2**	**0.3**	**0.1**	**5.6**	**1.7**
1452	neryl propionate	0.2	t	-	0.2	t	-	-
1484	**germacrene D***	**10.8**	**4.1**	**0.7**	**9.7**	**1.9**	**9.6**	**18.0**
1493	trans-muurola-4(14),5-diene	0.1	t	-	0.6	0.6	t	t
1500	**bicyclogermacrene***	**0.5**	**0.1**	**-**	**0.5**	**3.8**	**7.1**	**2.6**
1500	α-muurolene	0.3	t	-	0.2	-	t	0.3
1505	(E,E)-α-farnesene	1.0	0.6	2.2	0.3	0.3	0.5	1.0
1522	**δ-cadalene***	**0.3**	**t**	**t**	**3.5**	**1.3**	**0.4**	**t**
1529	kessane	t	-	-	-	-	-	t
1559	**germacrene B***	**-**	**0.6**	**t**	**-**	**0.2**	**0.8**	**-**
1559	43,109,65,238, sesquiterpene	0.5	t	-	0.5	3.1	2.2	1.0
1561	**(E)-nerolidol***	**0.1**	**-**	**-**	**-**	**0.3**	**0.4**	**1.7**
1573	isomer of 1159, sesquiterpene	0.3	-	t	0.2	1.2	1.0	0.4

KI	Compound	B4	B26	B21	B23	B10	B6	B7
1574	germacrene D-4-ol	-	0.1	t	t	0.1	0.3	0.4
1582	caryophyllene oxide	t	t	-	t	-	0.1	t
1594	caratol	-	-	0.1	0.9	-	t	-
1620	**germacrene D-4-ol isomer8**	**1.2**	**0.8**	**0.3**	**0.3**	**0.8**	**2.7**	**3.9**
1645	**cubenol***	**0.2**	**t**	-	**0.4**	**0.4**	**0.7**	**0.5**
1644	α-muurolol	0.3	t	-	0.2	0.3	t	0.2
1649	β-eudesmol	0.4	t	-	t	t	0.6	1.2
1652	α-cadinol	0.2	t	-	t	t	0.3	0.6
1678	41,159,177,220, sesquiterpene	-	t	-	2.1	-	-	t
1685	**germacra-4(15),5,10-triene-1-a**	**0.6**	**t**	-	**0.3**	**0.6**	-	**1.1**
1848	hexadecanal	t	t	t	-.1	t	t	t
1914	hexadecadienal	t	t	0.6	t	t	t	t
2111	phytol isomer	0.3	0.4	t	0.3	0.1	0.1	0.7
2135	55,83,159,320, diterpene	t	t	t	2.0	-	t	t

KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Vachellia x *ruthvenii* (*V. bravoensis* x *V. rigidula*) (Fabaceae: Mimosoideae) in Texas

David S. Seigler* and John E. Ebinger

Department of Plant Biology, University of Illinois, Urbana, IL 61801 (DSS)
Emeritus Professor of Botany, Eastern Illinois University, Charleston, IL 61920 (JEE)
*Correspondent:seigler@life.illinois.edu

ABSTRACT

Principal component (PCA) and principal coordinate analyses (PCO) suggest that *Vachellia bravoensis* and *V. rigidula* rarely hybridize. The putative hybrid shows a relationship to *V. bravoensis* because some leaves have a short rachis with 2 pinna pairs, and the pinnae have 5 to 12 pairs of leaflets. In contrast, the putative hybrid shows a relationship to *V. rigidula* with leaflets mostly longer and wider than in *V. bravoensis*, leaflets with obvious venation, and terminal leaflets of the pinnae being obovate. The hybrid between *V. bravoensis* and *V. rigidula* (*Vachellia* x *ruthveniii*) is described. Published on-line www.phytologia.org *Phytologia 97(2): 170-174 (July 1, 2015).* ISSN 030319430.

KEY WORDS: *Vachellia* x *ruthvenii, V. bravoensis* x *V. rigidula,* Fabaceae, hybrid.

The genus *Vachellia* Wight & Arn., which includes the species of *Acacia* s.l. with paired stipular spines and flowers that lack a floral disc, is represented by 60 species in the New World tropical and subtropical areas (Seigler and Ebinger 2005). Hybrids between a few New Word *Vachellia* species are occasionally encountered. These hybrids mostly involve species morphologically similar and probably related to *Vachellia macracantha* (Humb. & Bonpl. ex Willd.) Seigler & Ebinger that includes the ant-acacias and a few related taxa (Maslin and Stirton 1997, Ebinger and Seigler 1992, Seigler and Ebinger 1995). Hybrids between ant-acacia species and between ant-acacias and non-ant-acacias have been discussed by Janzen (1974), Ebinger and Seigler (1992), and Seigler and Ebinger (1995). Rarely do members of other species groups of *Vachellia* hybridize.

In August of 2001, while on a trip to the Chaparral Wildlife Management Area (CWMA) near Artesia Well, Dimmit and LaSalle Counties, Texas, an unusual specimen of *Vachellia* was pointed-out to John Ebinger and myself by Donald "Chip" Ruthven. Ruthven (presently at Matador Wildlife Management Area, Paducah, Texas) showed us this plant while we were studying the flora of the CWMA. The plant was 3 m tall with a crown nearly 4 m across. At that time the plant was dying, only a few mature, yellowing leaves were present on one of the branches, along with a well-developed basal sprout 20 cm tall with mature and developing leaves. We collected the basal sprout and the mature branches with dying leaves. Two years later the original plant had died and no other specimens of this unusual plant could be found on the CWMA.

The individual examined differed from both parents in many characters, but some characters were intermediate (Table 1). Its growth habit was similar to, but larger than most individuals of *Vachellia bravoensis* found on the CWMA and did not have the few upright gnarled stems common for *V. rigidula,* a species that typically dominates the limestone ridges (calcareous rises) at CWMA. No flowers or fruits were observed and no fruits were found on the ground surrounding the plant. This nearly dead individual was located on flat terrain in thorn-scrub vegetation. The parental species were present in the area along with many other scrubby species, mostly less than 4 m tall (Seigler et al. 2007). The present study was undertaken to examine the morphological differences of this probably hybrid individual and compare those characteristics with the probable parents: *Vachellia bravoensis* (Isely) Seigler & Ebinger and *V.*

rigidula (Benth.) Seigler & Ebinger. These two species are probably not closely related, but are sympatric throughout much of their geographic ranges in southern Texas and adjacent Mexico.

MATERIALS AND METHODS

Analyses were based on herbarium specimens of the putative parents and the hybrid from southern Texas (Appendix I). All specimens were collected by one or both authors and are presently deposited in the herbarium of the University of Illinois (ILL). Initially, the specimens were separated into probable taxonomic groups based on overall morphological similarity. These specimens were scored for seven characters (Appendix II). These data served as the source for principal component (PCA) and principal coordinate analyses (PCoA). Three or more measurements were made for each continuous character of each specimen. These values were then plotted to confirm that gaps in the data existed.

A PCA to identify groupings of the specimens examined was carried out. For this analysis, the data were first standardized and a correlation matrix, eigenvalues, and eigenvectors were calculated using NTSYS-pc verson 2.1 (Rohlf 2000). Eigenvectors were scaled by the square root of λ. The axes were rotated and the resulting loading values graphically represented as both two- and three-dimensional plots.

To carry out the PCoA analysis, Gower's resemblance coefficients were calculated (Legendre and Legendre 1983, Podani 1999, Dickinson 2000). The nature of each character was designated as binary, multistate, or quantitative descriptors and all characters were weighted equally (Dickinson 2000). The data matrix was transformed by the DCENTER algorithm using distances squared and eigenvectors and eigenvalues calculated with NTSYS-pc verson 2.1 (Rohlf 2000). Eigenvectors were scaled by the square root of λ. The resulting loading values were graphically represented as both two- and three-dimensional plots (Figure 1).

RESULTS

The analyses involved 15 specimens of *Vachellia bravoensis*, 13 of V. *rigidula* and one probable hybrid. The PCA based on seven characters (Appendix II), and a PCO based on Gower's similarity coefficients for species scored, proved to be similar (data not shown). In the PCA (Fig. 1), the first three principal components accounted for 47.9, 15.2, and 12.9 % of the variance (76% of the total variance). The two species are well separated on the first principal component (Fig. 1) and the putative hybrid is intermediate (Z01, *Vachellia* x *ruthvenii).*

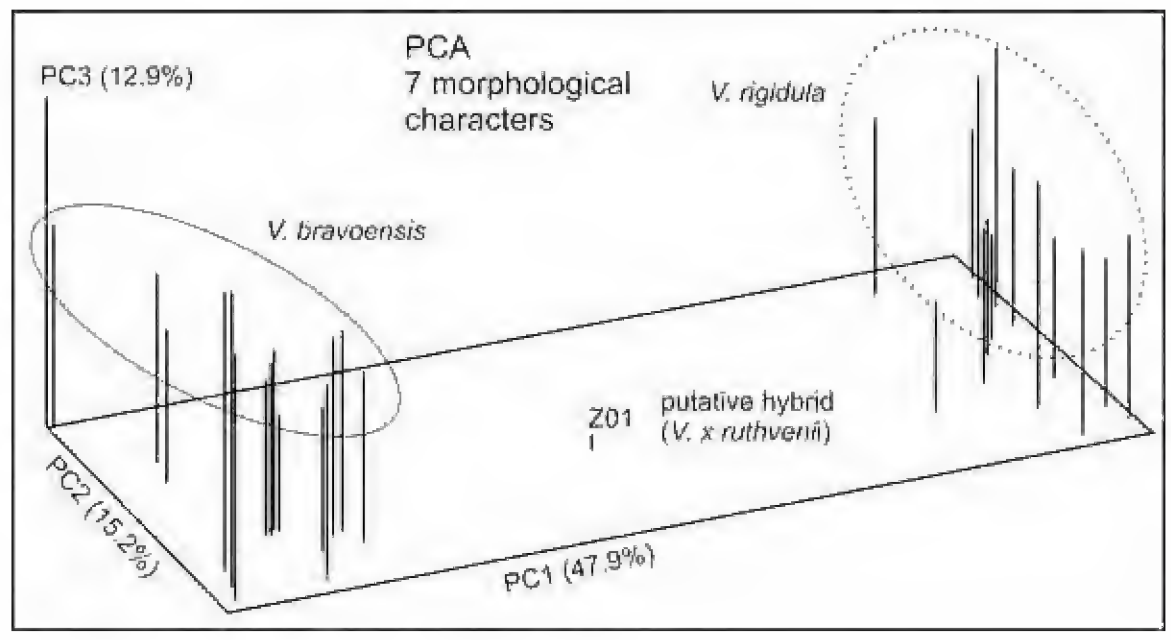

Figure 1. Plot of axis 1 v. 2 v. 3 for the principal components analysis using the seven characteristics (Appendix II) on 15 specimens of *Vachellia bravoensis*, 13 specimens of *V. rigidula*, and one specimen of the probable hybrid (*V.* x *ruthvenii*).

Rachis presence or absence (Rac), leaf venation (LVe), and leaflet length (LLe) (characters 1, 7, and 5) were most important for determining the component score of the first axis; petiole length (PLe), leaflet distance (LDi), and leaflet width (LWi) (characters 2, 4, and 6) were most important for determining the second axis.

DISCUSSION

Both parental species have a relatively narrow geographic range, being restricted to southern Texas and adjacent Mexico. For *Vachellia bravoensis,* we have found specimens from 25 southern Texas counties and the states of Coahuila, Hidalgo, Nuevo León, San Luis Potosí, and Tamaulipas, Mexico. *Vachellia rigidula*, in contrast, has a more extensive range being known from about 30 southern Texas counties and the Mexican states of Coahuila, Jalisco, Michoacán, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas, and Veracruz. We have observed numerous specimens of both species from throughout their ranges, and the specimen described below is the only specimen we have seen that appears to be of hybrid origin. The major differences separating the two parental species and the hybrid are shown in Table 1. The chromosome numbers of both parental species are known to be 2n = 26 (Turner & Fearing 1960). However, it was not possible to obtain this information from the proposed hybrid.

Vachellia* x *ruthvenii Seigler & Ebinger, **nothospecies nov.** (*Vachellia bravoensis* x *V. rigidula).*

TYPE: UNITED STATES. TEXAS: Dimmit Co.: Chaparral Wildlife Management Area, 2 miles NW of the laboratory building, 8 miles W of Artesia Wells, 20 August 2001, *D.S. Seigler, A. Kerber & J.E. Ebinger 15114* (ILL). (Figure 2). (Holotype: ILL, Isotype: ILL).

Shrub or small **tree** to 4 m tall; bark light gray to brown, smooth to shallowly furrowed; twigs dark purplish brown, slightly flexuous, lightly puberulent; short shoots commonly present above the stipular spines, 1-4 mm long, covered with acuminate stipules and old leaf bases; prickles absent. **Leaves** alternate, also clustered on the short shoots, 2-15 mm long; stipular spines light gray, symmetrical, terete, straight, woody, 0.2-3.0 x 0.2-1.2 mm near the base, lightly puberulent, persistent; petiole adaxially grooved, 2-7 mm long, lightly puberulent; petiolar gland solitary, located on the upper half of the petiole, sessile, circular to oblong, 0.2-0.7 mm long, apex depressed, glabrous; rachis adaxially grooved, 0-8 mm long, puberulent, glands absent; pinnae 1 to 2 pairs per leaf, 12-19 mm long, 4-7 mm between pinna pairs; paraphyllidia absent; petiolules 1.0-2.6 mm long; leaflets 5 to 12 pairs per pinna, opposite, 1.1-2.7 mm between leaflets, oblong (terminal pinna leaflets usually obovate), 3.5-7.5 x 1.2-2.2 mm, glabrous, lateral veins obvious, 1 to 3 veins from the base, base oblique, margins usually not ciliate, apex broadly acute to obtuse, usually mucronate, midvein subcentral. **Inflorescences, flowers, fruits, and seeds:** Not seen.

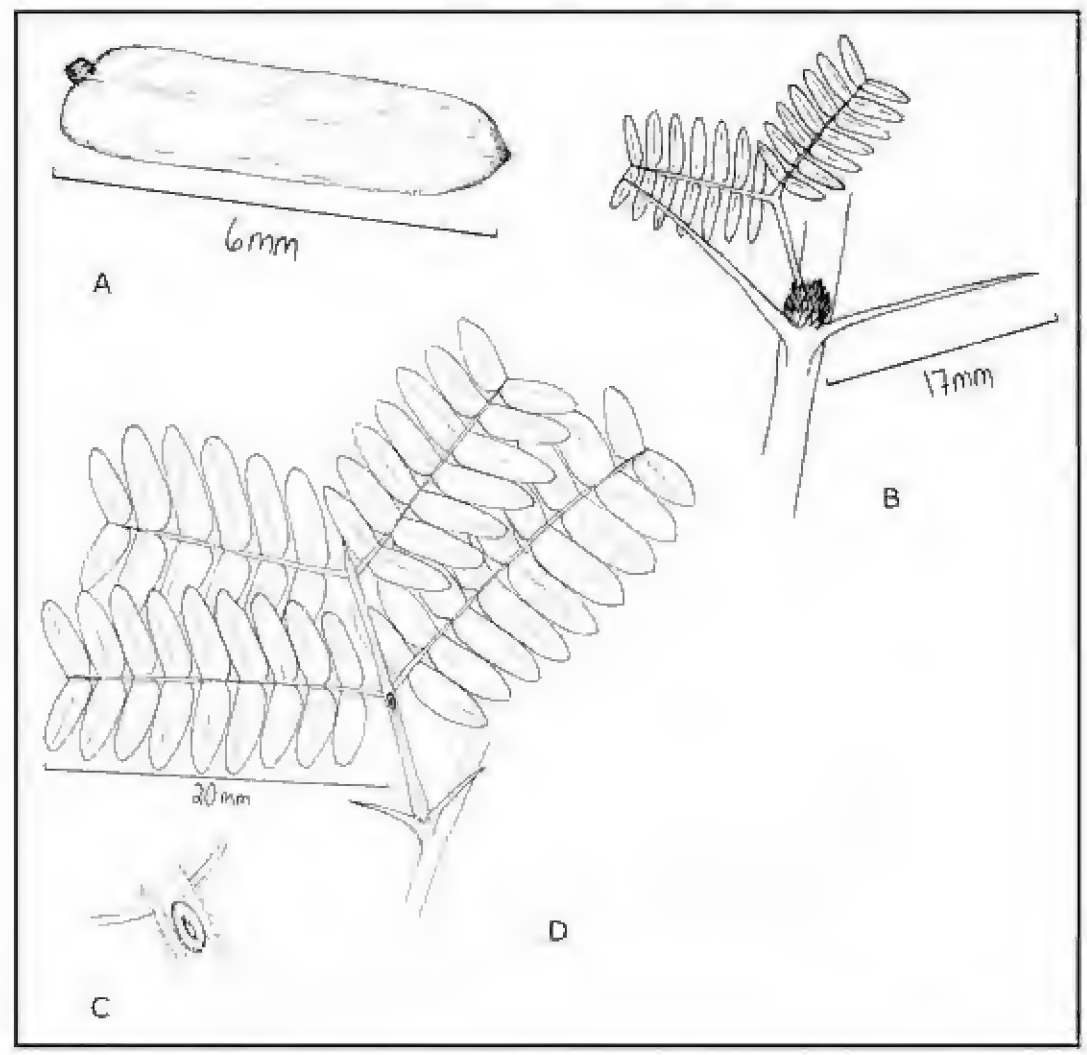

Figure 2. *Vachellia* x *ruthvenii* Seigler & Ebinger. A: Leaflet (abaxial surface); B. Node with stipular spines, short shoot and secondary leaf; C. Petiolar gland; D. Primary leaf with petiolar gland.

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Table 1. Characteristics that separate *Vachellia bravoensis*, *V. rigidula* and the hybrid *V. x ruthvenii*.

Characteristic	V. bravoensis	V. x ruthvenii	V. rigidula
rachis	mostly present	some present	absent
pinna pairs/leaf	1-3 (4)	1 to 2	1
petiolule length	0.4-1.0 mm	1-2.6 mm	1-3 mm
leaflet pairs/pinna	10 to 24	5 to 12	(2) 3 to 5
leaflets distance	0.5-1.1 mm	1.1-2.7 mm	1.5-5.0 mm
leaflet length	2.0-4.2 (5.0) mm	3.5-7.5 mm	(4) 6-13 (16) mm
leaflet width	0.6-1.1 mm	1.2-2.2 mm	3-6 (8) mm
leaflet margin	ciliate	not ciliate	not ciliate
leaflet venation	usually not obvious	obvious	obvious
terminal leaflet shape	linear to oblong	obovate	obovate to elliptic

Appendix I. Specimens scored for the principal component (PCA) and principal coordinate (PCoA) analyses.

Vachellia bravoensis: TEXAS: Atascosa Co.: S of Jourdantown, 29 Jun 1986, *D.Seigler & B.Maslin 12670*; road to San Miguel Power Plant, 10 Jul 1998, *D.Seigler & J.Ebinger 14331*. **Bexar Co.:** S-side of San Antonio, 22 May 1976, *D.Seigler, S.Saupe & H.Welt 10054*. **Dimmit Co.:** US 277, 26 miles E of junction of 57 and US 277, 16 Sep 1979, *D.Seigler & D.Young 11401*; Chaparral Wildlife Management Area, 22 May 2001, *D.Seigler & J.Ebinger 14889*, Chaparral Wildlife Management Area, 20 Aug 2001, *D.Seigler, A.Kerber & J.Ebinger 15116*; Chaparral Wildlife Management Area, 19 May 2005, *D.Seigler, J.Miller & B.Maslin 15931*. **Kinney Co.:** route 90, 4 miles W of Brackettville, 27 Jun 2002, *D.Seigler & J.Ebinger 15265*. **LaSalle Co.:** 3 miles W of Artesia Wells, 24 Aug 2001, *D.Seigler, A.Kerber & J.Ebinger 15135A*. **Maverick Co.:** US 277, 29 miles NW of Eagle Pass, 10 Jul 1998, *D.Seigler & J.Ebinger 14363*; 10 miles E of Eagle Pass, 27 Jun 2002, *D.Seigler & J.Ebinger 15268*. **McMullen Co.:** 1 mile N of Texas route 791, 11 May 1991, *D.Seigler, J.Ebinger, H.Clarke & K.Readel 13270;* S of Tilden, 10 Jul 1998, *D.Seigler & J.Ebinger 14336*. **Uvalde Co.:** Harris Ranch near Cline, route 90, 24 Jun 2002, *D.Seigler & J.Ebinger 15218*. **Webb Co.:** 7 miles N of junction of US 83 and I-35, on US 83, 21 May 1983, *D.Seigler, J.Kramer & E.Carreira 11958*.

Vachellia x ruthvenii: TEXAS: Dimmit Co.: Chaparral Wildlife Management Area, 2 miles NW of the laboratory, 28° 00' 34" N, 99° 26' 43" W, 20 Aug 2001, *D.Seigler, A.Kerber & J.Ebinger 15114*.

Vachellia rigidula: TEXAS: Atascosa Co.: 9 miles S of Jourdantown, route 1, 15 Sep 1979, *D.Seigler & D.Young 11381*; road to San Miguel Power plant, 10 Jul 1998, *D.Seigler & J.Ebinger 14323*. **Dimmit Co.:** Chaparral Wildlife Management Area, 19 May 2005, *D.Seigler, J.Miller & B.Maslin 15930*. **Frio Co.:** I-39, 1 mile SW of Bigfoot, 26 May 2001, *D.Seigler & J.Ebinger 15058*. **Jim Wells Co.:** 9 miles N of Alice, route 281, 6 Jun 1991, *D.Seigler, J.Ebinger, D.Clarke & K.Readel 13764*. **Kinney Co.:** 15 miles NE of Bracketville, route 334, 20 May 1976, *D.Seigler, S.Saupe & H.Welt 9931*. **McMullen Co.:** 3 miles E and 1 mile N of Tilden, 25 May 2001, *D.Seigler & J.Ebinger 15025b*. **San Patricio Co.:** 1 mile N of Sinton, route 77, 10 Jun 1980, *D.Seigler, P.Richardson & S.Thompson 11635*. **Starr Co.:** access road to Falcon Dam, 18 Feb 2004, *D.Seigler, J.Ebinger & L.Phillippe 15894*. **Val Verde Co.:** Langtry, 21 May 1976, *D.Seigler, S.Saupe & H.Welt 9955*; Pecos River Bridge, route 290, 16 Sep 1979, *D.Seigler & D.Young 11416*. **Webb Co.:** 45 miles NE of Laredo, route 59, 22 May 1976, *D.Seigler, S.Saupe & H.Welt 10038*. **Zapata Co.:** Arroyo Dolores, 25 miles N of Zapata, route 83, 18 Feb. 2004, *D.Seigler, J.Ebinger & L.Phillippe 15884*.

APPENDIX II: Characters used for PCA of *Vachellia bravoensis, V. rigidula*, and *V.* x *ruthvenii*.

1. Rachis (Rac) – 1. present, 2. absent;
2. Petiole length (mm) (PLe);
3. Leaflets pairs/pinna (LPi);
4. Leaflet distance (mm) (LDi);
5. Leaflet length (mm) (LLe);
6. Leaflet width (mm) (LWi);
7. Leaflet venation (LVe) – 1. 1ateral veins not obvious, 2 lateral veins obvious.

Taxonomy of the *Phacelia infundibuliformis* complex (Hydrophyllaceae)

Billie L. Turner
Plant Resources Center
The University of Texas
Austin TX 78712

ABSTRACT

The taxonomy of *Phacelia infundibuliformis* Torr. is reviewed, concluding that it is comprised of two infraspecific taxa: var. **infundibuliformis** from central Chihuahua, and Durango, Mexico as well as the Chianti Mountains (Presidio Co.) of Trans Pecos Texas, and var. **phanerandra** I.M. Johnst. of northeastern most Chihuahua, northwestern most Coahuila and closely adjacent Texas (Brewster and Presidio Counties). Published on-line www.phytologia.org *Phytologia 97(3): 175-178 (July 1, 2015).* ISSN 030319430.

KEY WORDS: Hydrophyllaceae, *Phacelia, P. infundibuliformis*

Phacelia infundibuliformis Torr., was first described from material reportedly collected by Bigelow and Wright from an "Overhanging rock on a mountain near Lake Santa Maria, Chihuahua." The author further notes that "Wright's specimens have a laxer habit, as well as larger and more membranous leaves than Bigelow's, probably from having grown in a shady place;" he also noted that the "species is remarkable for its funnel-form corollas." A var. *phanerandra* I.M. Johnst., with protruding stamens (vs not so) and funnel-form corollas was subsequently proposed, typified by material from northwesternmost Coahuila, Mexico, the latter taxon also occurring in closely adjacent Chihuahua and USA (Brewster Co.).

Phacelia infundibuliformis is of doubtful relationship within *Phacelia.* Indeed, Gillett (1968, p. 369), in his attempt to provide natural lineages in the Cosmanthus group of *Phacelia*, to which the present taxon might belong, commented that "the poorly known, rare, *P. infundubuliformis* Torr., of unassigned affinity, possibly a monotypic section, occurs in Brewster County, Texas...far to the east of several localities of the Cosmanthus phacelias." The latter collections belong to the var. **phanerandra**, Gillett presumably unaware of its proposal by I. M. Johnston, as noted below.

PHACELIA INFUNDIBULIFORMIS Torr., Rep. U.S. Mex. Bound. Surv. Bot. 144. 1859.
var. **infundibuliformis Fig. 1**

Annual herbs to 75 cm high, leafy throughout**. Mid-stems** moderately pubescent with glandular hairs 0.2-1.0 mm high. **Leaves** (the larger) pinnately dissected, lobed to the rachis, mostly 6-18 cm long, 4-9 cm wide, pubescent above and below. **Capitulescence**, 3-6 cm high, 3-5 cm across, branches markedly circinnate. **Pedicels** (flowering) 1-2 mm long. **Calyces** (flowering) 4-5 mm long, the 5 lobes linear-oblanceolate, parted to the base, pubescent with stiff, setose, hairs ca 1 mm long. **Corollas** 6-8 mm long, 'blue-lavender;" tubes glabrous, ca 6 mm long, the 5 lobes flared at the top, 4-6 mm across. **Stamens,** 5, included, separate to base, the filaments purple, glabrous; anthers yellow, ca 0.6 mm long. **Styles** not excurrent, ca 4 mm long, their branches ca 2 mm long. **Capsules** ovoid, ca 3 mm long, 2.5 mm wide, pubescent apically with both glandular (minute) and eglandular hairs. **Seeds,** 16-30 per capsule, brown, ca 4 mm long.

SPECIMENS EXAMINED:
MEXICO. CHIHUAHUA: 27 mi S of Cd. Chihuahua, route 45, 9 May 1959, *Correll & I.M. Johnston 21563* (LL-TEX); 10-15 mi SE of Nueva Casas Grandes. 9 May 1959, *Correll & I.M. Johnston 21696* (LL-TEX); "mouth of Majalca Canyon," 11 May 1959, *Correll & I.M. Johnston 21754* (LL-TEX). **DURANGO: Mpio. Nombre de Dios,** El Saltito, 25 Apr 1985, *Herrea 611* (TEX).

UNITED STATES. TEXAS: Presidio Co., Chinati Mtns. State Natural Area. Upper Tinaja Prieta fork of Pelillos Canyon, 29 51 28 N, 104 26 48 W, 4230 ft, 25 Mar 2005, *Lott 5456* (SRSC, TEX); "Capote Falls area," ca 30 13 N, 104 37 W, 3100 ft, mostly rocky calcareous soil, 16 Apr 1973, *M. C. Johnston et al. 10663* (TEX).

The two collections from Texas cited above were taken to be a novel species when first detected, my having relating them to *P. congesta.* My Academic Son, A. M. Powell (Turner 2015), called attention to their likely relationship to the poorly collected *P. infundibuliformis*, which proved to be the case.

var. **phanerandr**a I.M. Johnst., J. Arnold Arb. 24: 96. 1943.
As described for var. *infundibuliformis* but the anthers protruding from corollas, as noted below. The variety was not accounted for in Turner et al. (2003).

TYPE: **MEXICO. COAHUILA:** Sierra de las Cruces near Tinaja Blanca, 12 Mar 1942, *Stewart 2241* (GH).

Johnston also notes an additional specimen from Texas (Brewster Co., 14 mi E of Castolon, [w/o date] *Cutler 749*, GH). He also adds, "This variety occurs far to the east of the known stations for typical *G.* (sic) *infundibuliformis* and appears to be a geographic race distinguished by its protruding stamens. In other characters it agrees closely with the typical form of the species." I agree with his assessments.

SPECIMENS EXAMINED:
UNITED STATES. TEXAS: Brewster Co.: "north slope of mountain near Cottonwood Creek, along road between Terlingua and Basin, Big Bend National Park, 18 Apr 1961, *Correll & Rollins 23630* (LL-TEX). **Presidio Co.** "rare on igneous rock outcrops in shade of cliffs and nearby large cottonwoods, Ojito Adentro Spring, ca. 0.1 mi downstream from waterfall in box canyon, Big Bend Ranch SNA." 5 Mar 1991, *Carr 10971* (TEX); "North-facing cliffs, about 14 miles southeast of Redford, 14 May 1959, *Correll & I.M Johnston 21905* (LL-TEX).

Distribution of the two taxa, based upon published reports and specimens on file at LL-TEX, is shown in **Fig. 2**

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Turner, B.L. 2015. **All My Academic Children**. Texensis Publishing, Gruver, Texas, 134 pp.

Fig. 1. *Phacelia infundibuliformis var. infundibuliformis*

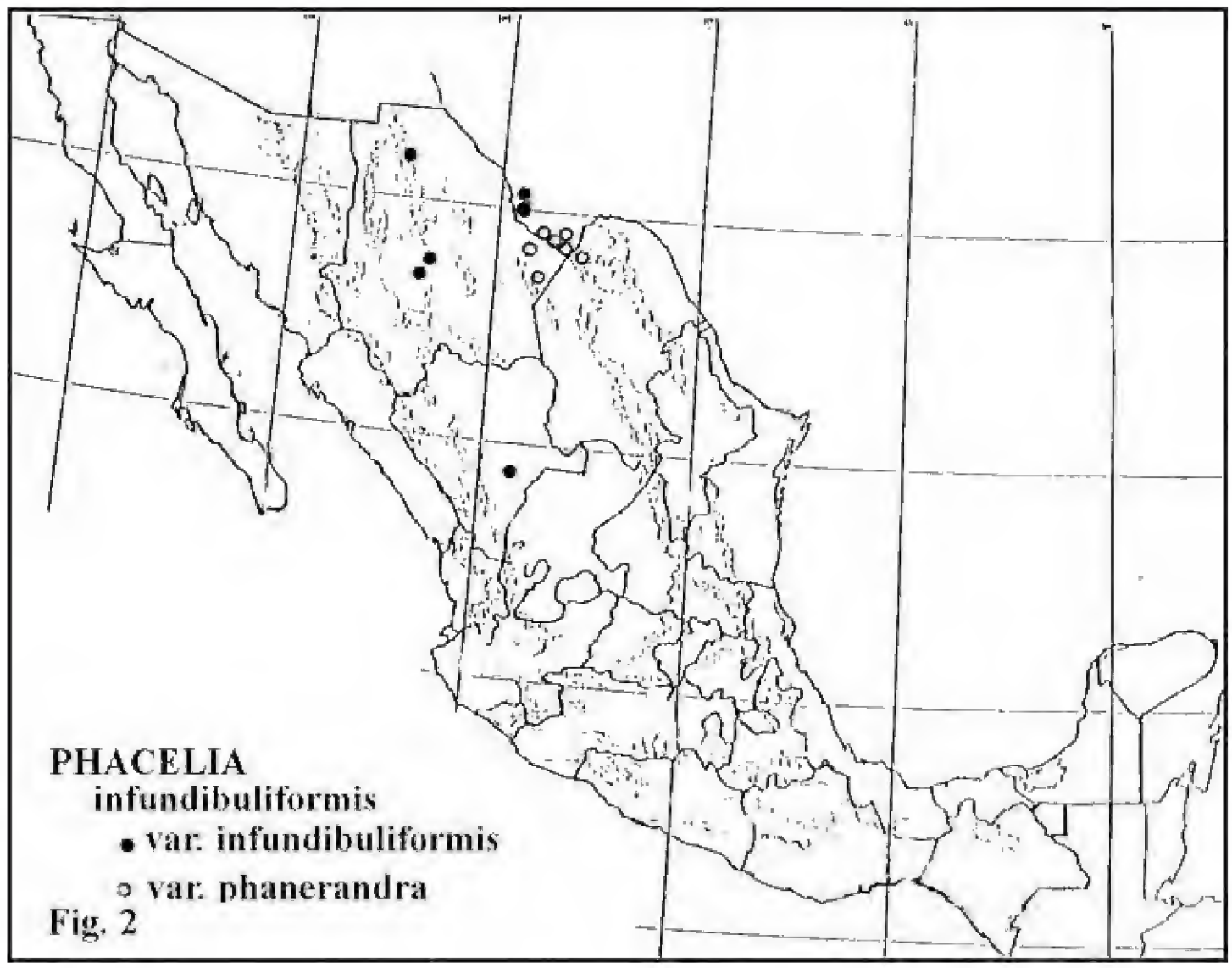

Fig. 2. *Phacelia infundibuliformis*, distribution of varieties.

Clarification of *Acacia multipinnata*, *A. paniculata*, *A. scandens* and *A. tenuifolia*

David S. Seigler[1] and John E. Ebinger[2]

[1]Department of Plant Biology, 505 S. Goodwin Ave., University of Illinois, Urbana, Illinois 61801
seigler@life.illinois.edu (author for correspondence)

[2]Emeritus Professor of Botany, Eastern Illinois University, Charleston, Illinois 61920, U.S.A.

ABSTRACT

Acacia paniculata and *A. tenuifolia* are morphologically distinct species that are difficult to distinguish; both are validly published, legitimate names. Type materials of *A. paniculata*, *A. scandens*, and *A. multipinnata* represent the same species. The oldest validly published, legitimate name for this latter group of species is *A. paniculata* Willd. (1806). Many authors have used the names *A. paniculata* and *A. tenuifolia* interchangeably and ambiguously. Although it is not the oldest name, in order to maintain nomenclatural stability and in anticipation of a proposal to conserve the name against *A. paniculata*, we have elected to use the name *A. multipinnata.* Published on-line www.phytologia.org *Phytologia 97(3): 179-186 (July 1, 2015).* ISSN 030319430.

KEY WORDS: Fabaceae, Mimosoideae, *Acacia multipinnata*, *Acacia paniculata*, *Acacia scandens*, *Acacia tenuifolia*, *Senegalia multipinnata*, *Senegalia paniculata*, *Senegalia scandens*, *Senegalia tenuifolia.*

A body of evidence including both morphological and molecular data have demonstrated that the formerly broadly circumscribed, pantropical genus *Acacia* Mill. (Leguminosae: Mimosoideae) is polyphyletic and presently is considered to be comprised of at least five genera: *Acacia*, *Acaciella*, *Mariosousa*, *Senegalia*, and *Vachellia*. Largely because of these factors, *Acacia* Mill. has been re-typified (McNeill et al. 2005, 2007) with an Australian species, *A. penninervis* Sieber ex DC. After two votes to accept this change by International Botanical Congresses (McNeill and Turland. 2011) this change is increasingly being adopted (Maslin, 2015).

Members of *Senegalia* are shrubs, trees, or lianas, unarmed or armed with prickles, but without stipular spines. The prickles usually are scattered, but less commonly are grouped in twos or threes, usually at or near nodes (Vassal 1972). The leaves are bipinnate and the petiole and primary rachis have sessile or stipitate glands of variable structure and position. Flowers possess a more-or-less tubular nectary below the usually stipitate ovary. Inflorescences are globose heads or spikes, often grouped into complex terminal pseudo-inflorescences (synflorescences). Pods are dehiscent, separating into two valves at maturity, or less commonly indehiscent or separating into indehiscent one-seeded articles. The seeds are uniseriate. *Senegalia* consists of approximately 100 taxa in the Americas (Ebinger and Seigler, unpublished data), as well as 69 in Africa, 43 in Asia, and two in Australia (Maslin et al. 2003, 2013 and World Wide Wattle, http: //www.worldwidewattle.com). Eight species occur in two or more areas.

Among species of *Senegalia*, the status of four names (*Senegalia multipinnata* (Ducke) Seigler & Ebinger, *S. paniculata* (Willd.) Killip ex Record, *S. scandens* (Willd.) Seigler & Ebinger and *S. tenuifolia* (L.) Britton & Rose) has been poorly understood. Although the original purpose of this study was to examine type materials and literature related to type materials for *Acacia paniculata* and *A. tenuifolia*, to determine if differences exist between the two species and to establish what should be the correct name

for these entities, as the study progressed it became clear that two other names, *A. multipinnata* and *A. scandens* had to be considered.

Senegalia multipinnata (Ducke) Seigler & Ebinger in Seigler, Ebinger & J. T. Miller, Phytologia 88: 60. 2006. TYPE: Brazil. Pará. "[L]ecta in regione Ariramba fluminis Trombetas," 10 Dec. 1910, *A. Ducke 11411* (lectotype, designated by Grimes [1992: 267], MG; isolectotypes, NY [1526], US).

Acacia paniculata Willd., Sp. Pl. 4: 1074. 1806 non J. F. Macbride (1919)
Mimosa paniculata Poir., Encycl., Suppl. 1(1): 74. 1810. nom. illeg. non Wendland (1798).
Acacia scandens Willd., Enum. Pl. 1057. 1809. nom. illeg. non Willdenow (1806)
Manganaroa paniculata (Willd.) Speg., Bol. Acad. Nat. Ci. (Córdoba) 26: 239. (pl. 241, 243). 1921
Acacia multipinnata Ducke, Arch. Jard. Bot. Río de Janeiro 4: 31. 1925
Senegalia cordobana Britton & Killip, Ann. New York Acad. Sci. 35: 143. 1936
Senegalia paniculata (Willd.) Killip ex Record, Trop. Woods 63: 6. 1940
Senegalia scandens Seigler & Ebinger in Seigler, Ebinger & J. T. Miller, Phytologia 88: 72. 2006

Senegalia tenuifolia (L.) Britton & Rose, N. Amer. Fl. 23: 118. 1928. TYPE: "Habitat in America calidiori" (lectotype, designated by Seigler et al. [2006: 74], t. 17 from Plumier [1755]). Note: According to Grimes (1992), t. 17 from Plumier (1755) was not published, but was seen by Linnaeus. This plate is located in the Codex Boerhavianus in the library of the Rijksuniversiteit Groningen.

Basionym: *Mimosa tenuifolia* L., Syst. Veg. 771. 1774
Acacia tenuifolia (L.) Willd., Sp. Pl. 4: 1091. 1806
Acacia julibrissin Sieb. ex Mart., "Herb. Fl. Bras." Flora 20(2). Beiblätter 109. 1837. nom. illeg. non Willdenow (1806)
Acacia clausseni Benth., London J. Bot. 1: 518. 1842
Acacia martinicensis K. Presl, Abh. Königl. Böhm. Ges. Wiss. Series 5. 3: 495. 1845
Acacia microcephala A. Rich. in Sagra, Hist. Phys. Cuba, Bot. Pl. Vasc. 4: 469. 1845. nom. illeg. non Macfadyen (1837)
Acacia tenuifolia (L.) Willd. var. *veraensis* Kitanov, Ann. Univ. Sofia Fac. Biol. 64(2): 60. 1972

The name *A. paniculata* Willd. (1806) is based on type material at B. There are two specimens with Cat. No. 19157, both Hoffmannsegg collections (Grimes 1992). Grimes lectotypified *A. paniculata* based on the flowering specimen; the fruiting specimen is a member of the genus *Anadenanthera*. Grimes (1992) also located type materials for *A. tenuifolia*, which was later lectotypified (Seigler et al. 2006).

In 1806, Wildenow retained *Acacia tenuifolia* (L.) Willd. (based on *Mimosa tenuifolia* L.) and described *A. paniculata* as a new species. Subsequently many authors have confounded the concept of *A. paniculata* with non-synonymous taxa. As a part of our continuing study of this difficult group of species, we examined type material for *A. paniculata* and compared morphological characters to those of *A. tenuifolia* based on Grimes (1992) who obtained data for the type materials of that species. In contrast to the type of *A. tenuifolia*, the type specimen of *A. paniculata* lacks anther glands, the midribs of leaflets are more-or-less central, and leaves of the type specimen possess 30-40 pairs of pinnae. At the same time, we examined the type of *Senegalia scandens* (Willd.) Seigler & Ebinger (Seigler et al. 2006) at B and, in our judgement, this specimen is identical in defining characteristics to that of *A. paniculata*. In contrast, specimens of *A. tenuifolia* typically have anther glands, fewer than 20 (but occasionally up to 28) pairs of pinnae and the leaflet midribs are marginal. The description in Linnaeus (1753) indicates that the species has "partialibus viginti-jugatis: propriis multijugatis."

Plants congruent with the types of *Acacia paniculata* and *A. tenuifolia* occur from Mexico to southern South America. Those of *A. tenuifolia*, but not *A. paniculata*, are found in the Caribbean area (Ebinger and Seigler, unpublished data). In all probability, authors before 1806 (for instance, Houttuyn [1779] and Lamarck [1783]) attributed specimens of these species to *Mimosa tenuifolia* L. After 1806, taxonomists varied in their interpretation of these two species. Based on admittedly limited material, Bentham (1842) considered *A. paniculata* to be similar to a specimen from St. Lucia that was almost certainly *A. tenuifolia*. By the time of his 1875 monograph, Bentham considered *A. tenuifolia* (L.) Willd., *M. tenuifolia* L., *M. paniculata* Poir. and *M. paniculata* West in Vahl (1807) to be synonyms of *A. paniculata* Willd. and applied the "Kew Rule," choosing the name *A. paniculata* (Grimes 1992). In the following year, Bentham (1876) included a description of *A. paniculata* with "costa submarginali" and "pinnis 10-20 jugis," corresponding to *A. tenuifolia,* along with plate 101 that has anthers that lack glands and leaflets with more-or-less central midribs. These characters correspond to those of *A. paniculata*, although the number of pinna pairs corresponds to *A. tenuifolia*. Nonetheless, probably due to the influence of Bentham, subsequent authors frequently considered *A. paniculata* to be conspecific with *A. tenuifolia*. However, Britton and Rose (1928) transferred *M. tenuifolia* to *Senegalia* to form *Senegalia tenuifolia* (L.) Britton and Rose without mentioning *A. paniculata*.

Additional confusion occurred because of a Caribbean species *Mimosa paniculata* (West 1793), a nomen nudum from the Danish West Indies. This was later published as a new name, *M. paniculata* West ex Vahl (1807) that proved to be an illegitimate name (non Wendland [1798]). Afterward, *M. paniculata* West ex Vahl served as the basis for the new name *Acacia westiana* DC. (1825) and later as a new combination *Senegalia westiana* (DC.) Britton and Rose (1928). Vahl (1807) observed that *M. paniculata* West ex Vahl is different from *M. tenuifolia* L., although de Candolle (1825) conversely considered that *A. westiana* might be *M. tenuifolia*. To further complicate things, G. Don (1832) considered *M. paniculata* West ex Vahl to differ from *A. paniculata* Willd., but considered *A. paniculata* Willd. possibly to be the same as *M. tenuifolia* L. Subsequently, *S. westiana* has been recognized as a legitimate species by several authors including Britton and Rose (1928); Acevedo and Strong (2012); Bentham (1875) and Rico-Arce (2007). We consider this taxon to be a synonym of *A. riparia* Kunth (1823) (= *S. riparia* (Kunth) Britton and Rose in Britton and Killip (1936).

Because plants that correspond to the type of *Acacia paniculata* do not occur in the Caribbean area, investigators who reported the presence of *A. paniculata* or *A. tenuifolia* in their study areas such as Acevedo and Strong (2012); Duss (1897); León and Alain (1951); Stehlé (1946) (as *Acacia tenuifolia*) and Stehlé et al. (1949) (as *A. paniculata*) actually examined *A. tenuifolia*. Howard (1988) considered *A. tenuifolia* and its synonyms to be the species common to the Lesser Antilles. Taxonomists who studied Caribbean plants worked with *A. tenuifolia* (L.) Willd. and not with *A. paniculata* Willd.

Information concerning salient features provided by the authors who studied *Acacia tenuifolia* and *A. paniculata* in Central and South America sometimes makes it possible to determine the identity of the materials used. Based on identification of herbarium materials and species ranges, some investigators probably had specimens of both *A. paniculata* and *A. tenuifolia* among their study materials and did not distinguish the two species. The characters cited by several investigators indicate that they worked with *A. tenuifolia*. For example, Ducke (1925) erroneously observed that "Le vrai *Acacia paniculata* a cependant les feuilles moins longues et les anthers (dans les boutons) couronnées d'une glande de couleur foncée" and cited *Kuhlmann 3233* (RB) from Amazonas, Brazil, and material of the states of Minas Geraes and Ceará. His description indicates that the material to which he referred was *Senegalia tenuifolia.* Barroso (1964); Cárdenas and Martino (2001); da Silva (1990); Macbride (1943); Madsen (1990); McVaugh (1987); Woodson and Schery (1950) and Zamora (1991) all worked with *S. tenuifolia*. Rico-Arce (2001a, b and 2007) considered the materials she examined to be *A. tenuifolia*, but reported eglandular stamens. Although specimens of *A. tenuifolia* normally have anther glands, their absence is sometimes a function of age and the condition of flowers (Grimes 1992). Pulle (1940) correctly noted that *A. paniculata* lacked

anther glands and usually had 20-30 pairs of pinnae suggesting that he worked primarily with specimens of *A. paniculata*. Spegazzini (1921) proposed the genus *Manganaroa* largely based on the presence of anther glands. Based on his extensive descriptions, the materials examined were almost certainly *A. tenuifolia* (p. 239), which he designated as *Manganaroa paniculata* (Willd.) Spegazzini.

In contrast to the above examples, it is not always possible to determine whether the material examined was *Acacia paniculata* or *A. tenuifolia*, or perhaps other species. A number of investigators considered *A. paniculata* to be a synonym of *A. tenuifolia*: Barros (2011); Barros and Morim (2014); da Silva (1990); de Queiroz (2009); Forero and Romero (2009); Funk et al. (2007); Grimes (1992); Jørgensen and León (1999); Rico-Arce and Fonseca (2005); and Ribeiro (2012). Other workers have used the name *A. paniculata* without mentioning synonyms or *A. tenuifolia*: Barbosa et al. (2004); Calderón and Standley (1941); Chodat and Hassler (1904); Hassler (1898); Killeen et al. (1993); Lewis and Owen (1989); Rodal and Nascimento (2002); and Sprengel (1826).

As an additional part of our examination of relevant type material in the Willdenow collections at B, we discovered that the type of *A. scandens* Willd. (1809), an illegitimate name (non Willdenow [1806]), (B-W [bc] B-W 19194-010) [bc = barcode] was nearly identical to that of *A. paniculata* and represents the same species. As a further part of our study of *Senegalia,* we also examined isotypes of *A. multipinnata* and found that the specimens of all three species lacked anther glands, the leaflets had central venation, more than 20 pairs of pinnae (occasionally as few as 15 on leaves near the inflorescence) and, thus, possessed the major characters of *A. paniculata*. Upon microscopic examination, the types were nearly identical in pubescence and other observable features and represent material of a single species.

Acacia multipinnata Ducke is a widespread species of moist evergreen primary tropical forests and disturbed primary and secondary forests from sea level to 1,000 m in southern Mexico, Costa Rica and Panama south to Bolivia and western Brazil. It also occurs in Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, and Venezuela. In addition to the lectotype, a number of syntypes are known: *A. Ducke 10457, R. Spruce 494* (K [bc] K 000117769), and *J. G. Kuhlmann 17487* (K [bc] K 000117768, U [bc] U 0007913, US [bc] US 01108075), S [bc] S-R-8520.

Senegalia multipinnata (*Acacia multipinnata*) has previously been considered synonymous with *A. paniculata*. Ducke (1925) observed that specimens of *S. multipinnata* were collected by himself (*Ducke 11411* and *10457*), Spruce (*Spruce 494*), and Kuhlmann (*Kuhlmann 17487*) and "sub nomine *A. paniculata* distributa." Based on our examination of the type specimen of *A. paniculata* and comparison to type materials of *A. multipinnata*, we concur that *A. paniculata* is synonymous with *A. multipinnata*, but as noted above, *A. tenuifolia* should not be considered a synomym.

The name *Acacia multipinnata* has been widely used in the taxonomic, ecological, biological, and chemical literature from the time of its publication in 1925, primarily by South American investigators: Brako and Zarucchi (1993) (as a synonym of *Acacia tenuifolia*); Clarke et al. (2001); da S. Ribeiro (1999); Da Silva (1990); Ducke (1925); Forero and Romero (2009); Jørgensen and León (1999); Lemée (1951); Madsen (1990) and Rico-Arce (2007).

Irwin (1966) segregated *Acacia paniculata* into five groups. Group A included plants with a marginal costa. We have examined two of the specimens he cited [*A. C. Smith 2388, 3445*], both from British Guiana, and found them to be *A. tenuifolia*. Irwin considered the type of *A. paniculata* to belong to this group. He placed *A. multipinnata* in synonymy with *A. paniculata* with no comment. In groups B and C, specimens had subcentral leaflet midribs. Several of his specimens of groups B and C that we have been able to examine are *A. multipinnata* (Upper River Sapary, *Krukoff 1146*; Rio Trombetas, *Ducke 11411*, the lectotype of *A. multipinnata* (Grimes 1992); Esperanca, *Ducke 1026*; Amazonas, Tres Casas, Mun. Humayta, *Krukoff 6340*; and Bolivia, near La Paz, 700-800 m, *Krukoff 10160*). The species in

Irwin's Group D and E represent an undescribed species similar to *Senegalia podadenia* (Britton and Killip) Cárdenas. Specimens of Groups D and E that we have examined are: Amapá: Rio Araguari, *Pires, Rodrigues & Irvine 51192*, and Rio Oiapoque, *Irwin, Pires & Westra 48464*. Grimes (1992) lectotypified *A. paniculata*, and located type materials for *A. tenuifolia*, which was later lectotypified (Seigler et al. 2006). Grimes considered these two species and *A. multipinnata* to be conspecific, lectotypified both *A. multipinnata* and *A. paniculata*, and placed them into synonymy under *A. tenuifolia.*

We conclude that the type materials of *Acacia paniculata*, *A. scandens*, and *A. multipinnata* represent the same species. The oldest validly published, legitimate name for this group of species is *A. paniculata* Willd. (1806). Many authors have used the names *A. paniculata* and *A. tenuifolia* and interchangeably and ambiguously. In selected cases, regardless of the name employed, it is possible from cited distributions and from morphological characters in descriptions to ascertain the probable identity of the materials examined by the authors, but in many other cases the identity of the materials studied remains an open question. *A. tenuifolia* is clearly distinct and should not be considered as a synonym of *A. multipinnata*, *A. paniculata* or *A. scandens*. Because of this confusion we have elected to use the name *A. multipinnata*, although it is not the oldest name, in order to maintain nomenclatural stability in anticipation of a proposal to conserve the name against *Acacia paniculata.*

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Allopatric hybridization and introgression between *Juniperus maritima* R. P. Adams and *J. scopulorum* Sarg. II. Additional Evidence from nuclear and cpDNA genes in Montana, Wyoming, Idaho and Utah

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA, email Robert_Adams@baylor.edu

ABSTRACT

A previous study using data from nrDNA (ITS), maldehy, and petN-psbM (cpDNA) confirmed that allopatric hybridization is occurring at Wallowa, OR, eastern WA, and southeastern BC into western Montana. nrDNA was found to be of less use in detecting hybrids than a single copy nuclear gene (SCN), maldehy. This might to be due to concerted evolution in nrDNA or relictual effects from ancient speciation. The uniform presence of either *J. maritima* cpDNA in western BC and WA or *J. scopulorum* cpDNA in eastern BC, WA, OR, and MT suggests allopatric introgression by air-borne pollen. *Juniperus* trees in the study area can be divided into roughly four zones: 1. typical *J. maritima*: Puget Sound, Vancouver Island, islands in the Strait of Georgia, and western BC; 2. intermediate trees: eastern WA, Wallowa, OR, southeastern BC, and western MT; 3. trees introgressed from *J. maritima* into *J. scopulorum*: Montana and northeastern Wyoming; 4. mostly typical *J. scopulorum*: south eastern ID, Utah and southward in the Rocky Mtns. Published on-line **www.phytologia.org** *Phytologia 97(3): 187-199 (July 1, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus maritima, J. scopulorum, nrDNA, maldehy, petN-psbM,* leaf terpenoids, hybridization, introgression, Pleistocene refugia, recolonization, Wisconsin glaciation.

Recently, I published an extensive analysis of reputed allopatric hybridization and introgression between *Juniperus maritima* and *J. scopulorum* (Adams, 2015). The overall trend was the presence of *J. maritima* in the northwestern US and British Columbia (BC) with intermediate trees (hybrids and back-crosses) in eastern WA and OR, eastern BC and Kalispell, MT (Fig. 1.). The cp marker (petN-psbM) gave the clearest delineation between the taxa. All the intermediate trees had *J. scopulorum* cpDNA (via pollen), with only two intermediate trees having *J. maritima* cp DNA (Wallowa, WO, Fig. 1). *Juniperus maritima* nrDNA (ITS) was found in all trees, except for two putative hybrids at Williams Lake, BC (WL, Fig. 1) and two hybrids at Fairmont Hot Springs, BC (FH, Fig. 2). Maldehy appeared to be a more sensitive indicator of hybrids than nrDNA, in that several trees contained heterozygous maldehy from *J. maritima and J. scopulorum* (Fig. 1).

However, I was surprised to find no typical *J. scopulorum* (by all three DNA markers) in Wallowa, eastern Washington, or Kalispell, MT. Five reference *J. scopulorum* trees from Utah and New Mexico were pure *J. scopulorum* (by the three DNA markers) (Fig. 1). Left unanswered was the extent of introgression eastward into *J. scopulorum* in Montana, Wyoming, Idaho and Utah. The purpose of the present paper is to extend the analyses of the previous work (Adams, 2015) to include additional *J. scopulorum* trees from Montana, Wyoming, Idaho and Utah to further analyze the eastern extent of introgression.

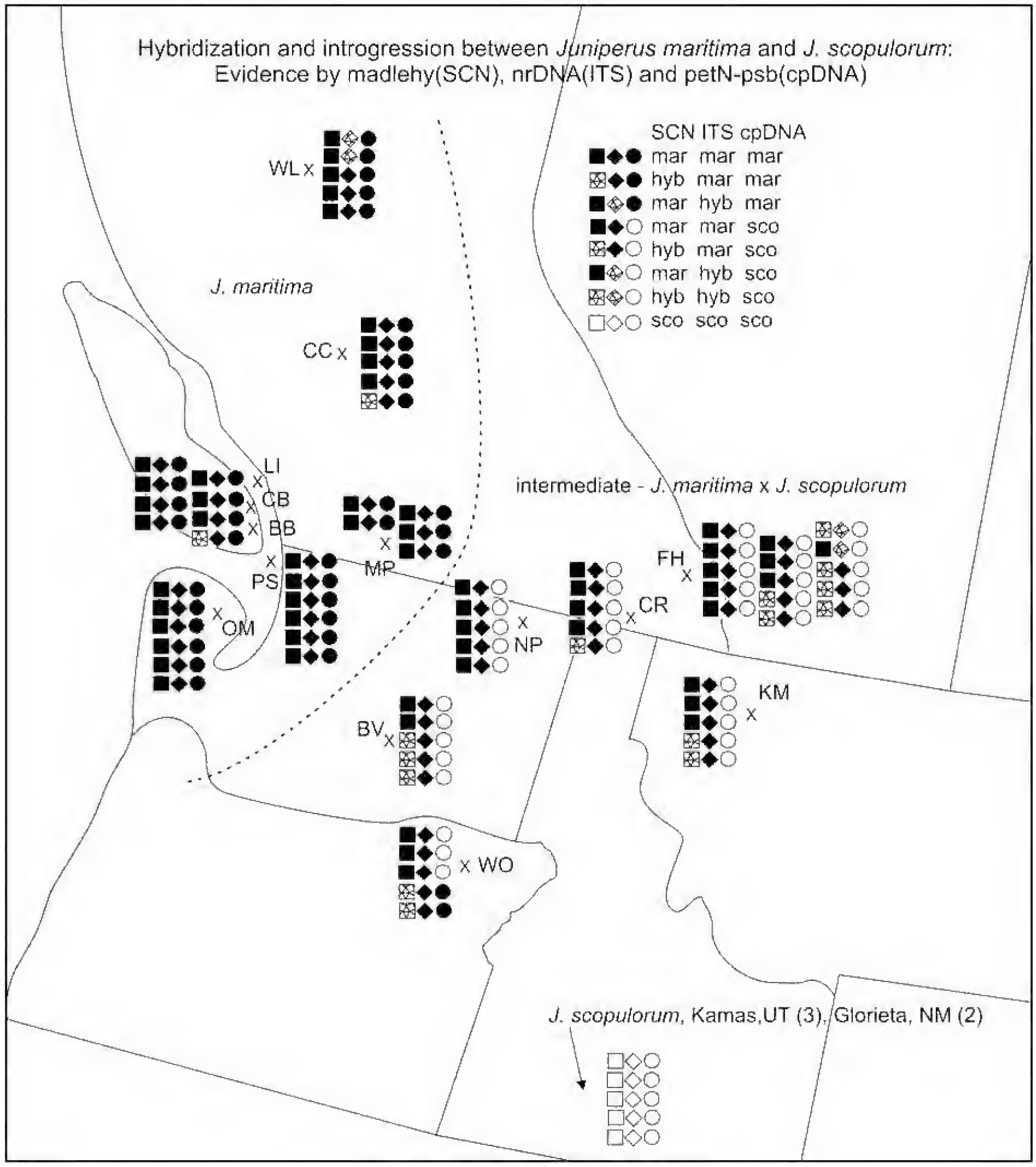


Figure 1. Combined classification of trees based on maldehy, nrDNA, and cpDNA sequence data. From Adams, 2015.

MATERIALS AND METHODS

Plant material: (species, population acronym, location, vouchers):

J. maritima: BB, Brentwood Bay, Vancouver Island, BC, *Adams 11056-11058;* CB, Cowichan Bay, Vancouver Island, BC, *Adams 11061-11063*; LI, Lesqueti Island, BC, *Adams 11064-11066*; Vancouver Island, BC; PS, San Juan Island, WA, *Adams 11067, 11068*; Whidbey Island, WA, *Adams*

11075; Fidalgo Island State Park, WA, *Adams 11076*; Skagit Island, WA, *Adams 11077-1178* (*11077* is the national big tree for *J. scopulorum*, but should be the *J. maritima*, national big tree); WL, Williams Lake, BC, *Adams 13436-13440*; Cache Creek, BC, *Adams 13431-13435*; MP, Manning Park, BC, *Adams 13426-13430*; Olympic Natl. Forest, WA, *Adams 11999-12004*.

J. scopulorum: Reference, Kamas, UT, *Adams 10895-10899,13887-13891,* and Glorieta Pass, NM, *Adams 10933-10935*.

Putative *J. maritima x J. scopulorum*: CR, Creston, BC, *Adams 14026-14030*; FH, Fairmont Hot Springs, BC, *Adams 13421-13425, 14001-14010*; *Adams 14001-14010*; Northport, WA, *Adams 14031-14035*; BV, Beverley, WA, *Adams 14036-14040*; WO, Wallowa Mtns., OR, *Adams 11935-11939*; KM, Kalispell, MT, *Adams 12995-12999*;

Additional *J. scopulorum* from the northern Rocky Mtns.: Soda Springs, ID, *Adams 7063-7066*, Moorcroft, WY, *Adams 10876-10878*; Big Sky, MT, *Adams 10882-10884*; Butte, MT, *Adams 10885-10889*. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used), 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. See Adams (2015) for maldehy primers and PCR conditions.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Minimum spanning networks were using PCO3d and MINSPAN software (Adams et al., 2009; Adams, 1975; Gower, 1966, 1971; Veldman, 1967).

RESULTS AND DISCUSSION

DNA sequencing gave: nrDNA (1270bp), with 5 substitution differences between the reference populations of *J. maritima* (BB) and *J. scopulorum* (KU, GN); petN-psbM (828bp), 8 nucleotide differences plus a 7 bp indel; maldehy (522bp, *maritima*; 529 bp, *scopulorum*), 2 differences plus a 7 bp indel. Each of these sequences displayed fidelity in the reference populations except for two trees in the Kamas, UT population (Table 1). Based on these distinct differences, an effort was made to classify each plant as to species or hybrid for maldehy and nrDNA. Of course, it may be that some positions will be heterozygous by chance, from relictual speciation or from ancestral hybridization.

Table 1 shows the classification of 101 individuals for each of these three gene regions. All of the samples from the Puget Sound - Strait of Georgia - Olympic Mtns, plus Manning Park were uniformly classified as *J. maritima*, except for *11063*, Cowichan Bay, Vancouver Island, for which maldehy was heterogenic for both substitutions, and, thus, classified as a hybrid. nrDNA was slightly more conserved in detecting hybrids, with 10 hybrids and 2 back-crosses, compared to maldehy that found 16 hybrids (Table 1). In only one case (13421, Fairmont Hot Springs, BC) did nrDNA and maldehy classify the same tree as a hybrid. However, another case (10889, Butte, MT) had *J. maritima* maldehy and intermediate (or backcrossed) nr DNA (Table 1). The conserved nature of the multi-copy nrDNA (up to

millions of copies per cell) might be due to concerted evolution (Liao, 1999). The latter author argues that because rRNAs are structural molecules, multiple gene copies are necessary to supply the demand for ribosomal subunits in the cell. Since these sub-units function only when assembled into a large complex, homogeneity of rRNAs is critical for regular, functional ribosome assembly and translation to function normally. Liao (1999) concludes that "a possible biological function of concerted evolution is to maintain homogeneous gene copies in a family so that homogeneous transcripts can be produced." However, concerted evolution is thought to be a slow process over numerous generations. Hybrids would seem likely to be heterozygous for both parents nrDNA.

The distribution of cpDNA (petN-psbM) shows a clear trend (Fig. 2) with *J. maritima* petN confined to the western BC, Vancouver Island - Puget Sound, WA, and Olympic Mtns., WA, with the exception of two trees in the Wallowa Mtns., OR (WO). Likewise, *J. scopulorum* petN is confined to southeastern BC, eastern WA, Kalispell, MT (KM) and 3 trees in the Wallowa Mtns., OR (Fig. 3). The pattern is suggestive of *J. scopulorum* pollen flow carrying petN towards the northwest. Four nrDNA hybrids were found in the Williams Lake (WL) and Fairmont Hot Springs (FH) populations (Fig. 3) and another four plus two backcrosses in the intermediate (MT, WY) zone (Fig. 3). Mostly typical *J. scopulorum* nrDNA occurred from Idaho southward (Fig. 3).

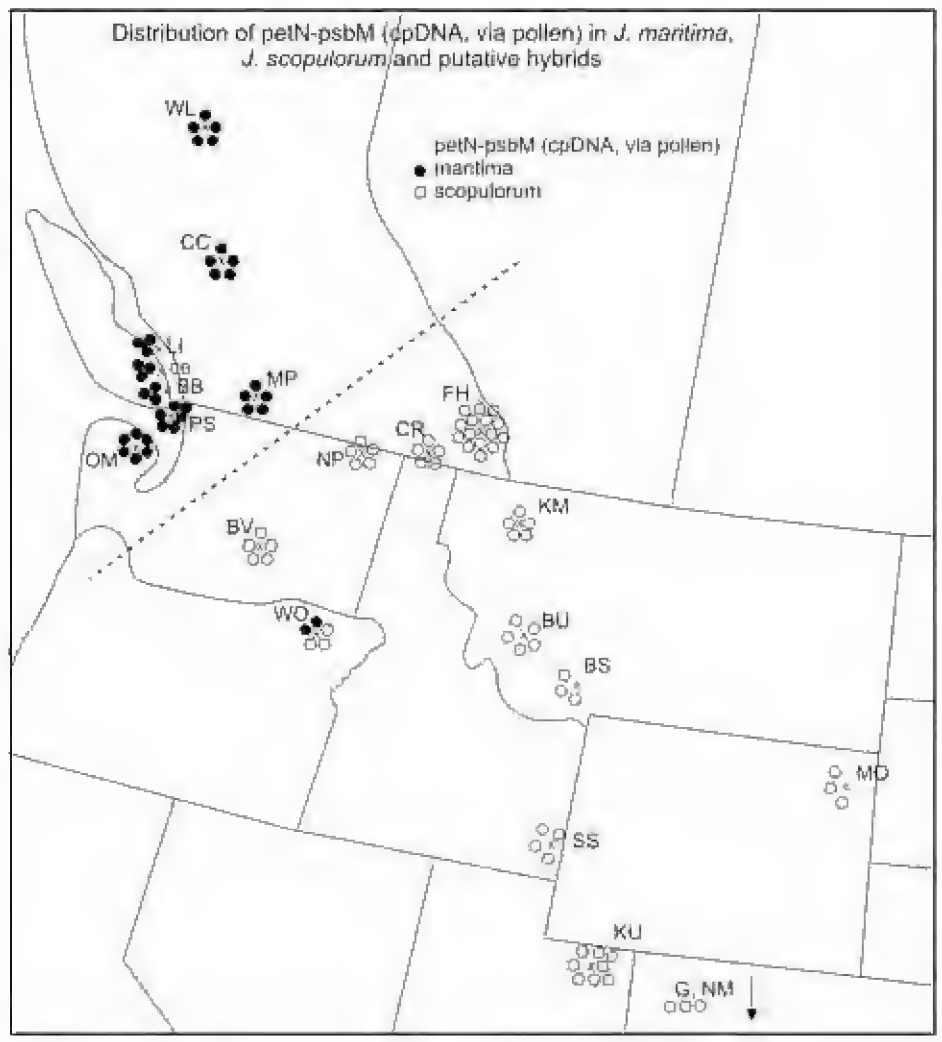


Figure 2. Classification by cpDNA (via pollen). Note the sharp break in western BC in petN.

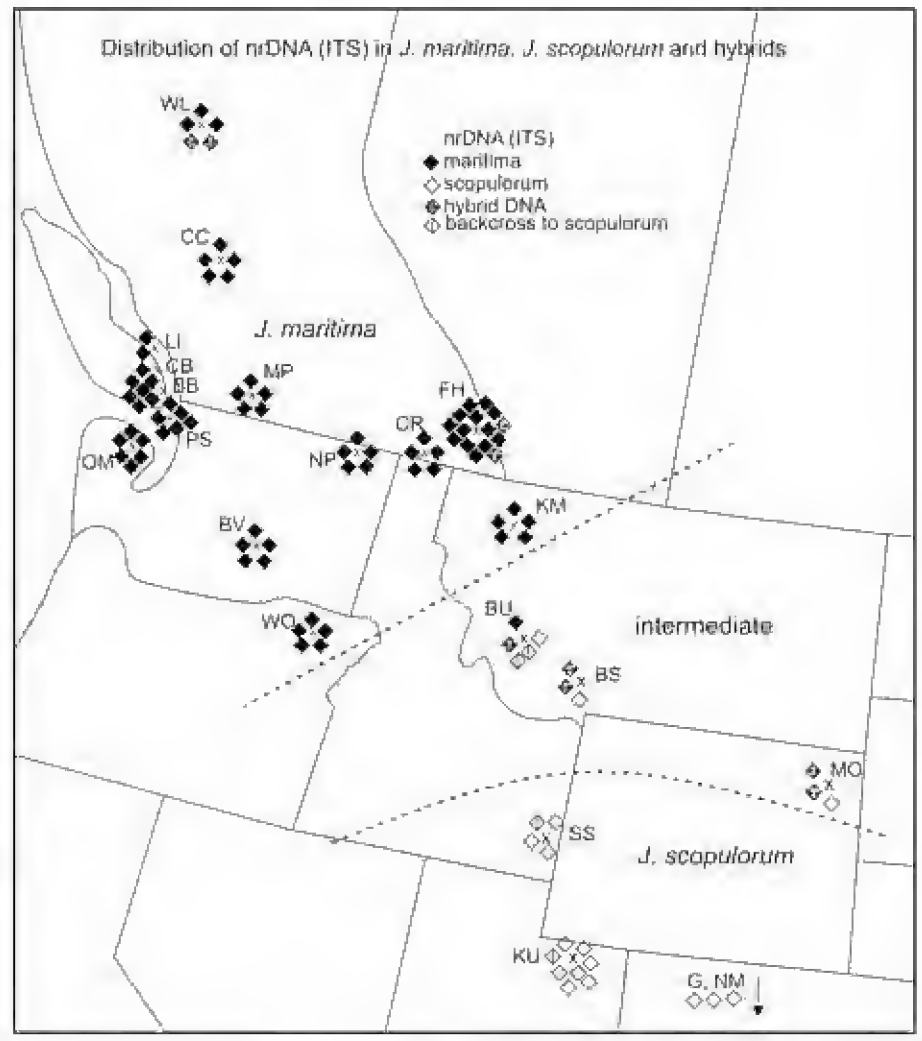


Figure 3. Classification by nrDNA. Note the zone of intermediate nrDNA in Montana and Wyoming.

The distribution of maldehy types gives an interesting comparison to nrDNA and petN (Fig. 4). Homogenic *J. scopulorum* maldehy trees were confined to southern Montana and southward in the Rocky Mtns. However, homogenic *J. maritima* maldehy individuals were widespread across the study area (Fig. 4). One hybrid was found in the CC (Cache Creek) population, whereas all the other hybrid maldehy plants were in eastern BC, Beverly, WA (BV), Wallowa Mtns., OR (WO) and Kalispell, MT (KM). Kalispell (KM) and Wallowa (WO) appear to be at the northwestern boundary of typical *J. scopulorum*. The area of intermediates (Fig. 4) is similar, but not identical, to that of the nrDNA intermediates (Fig. 3).

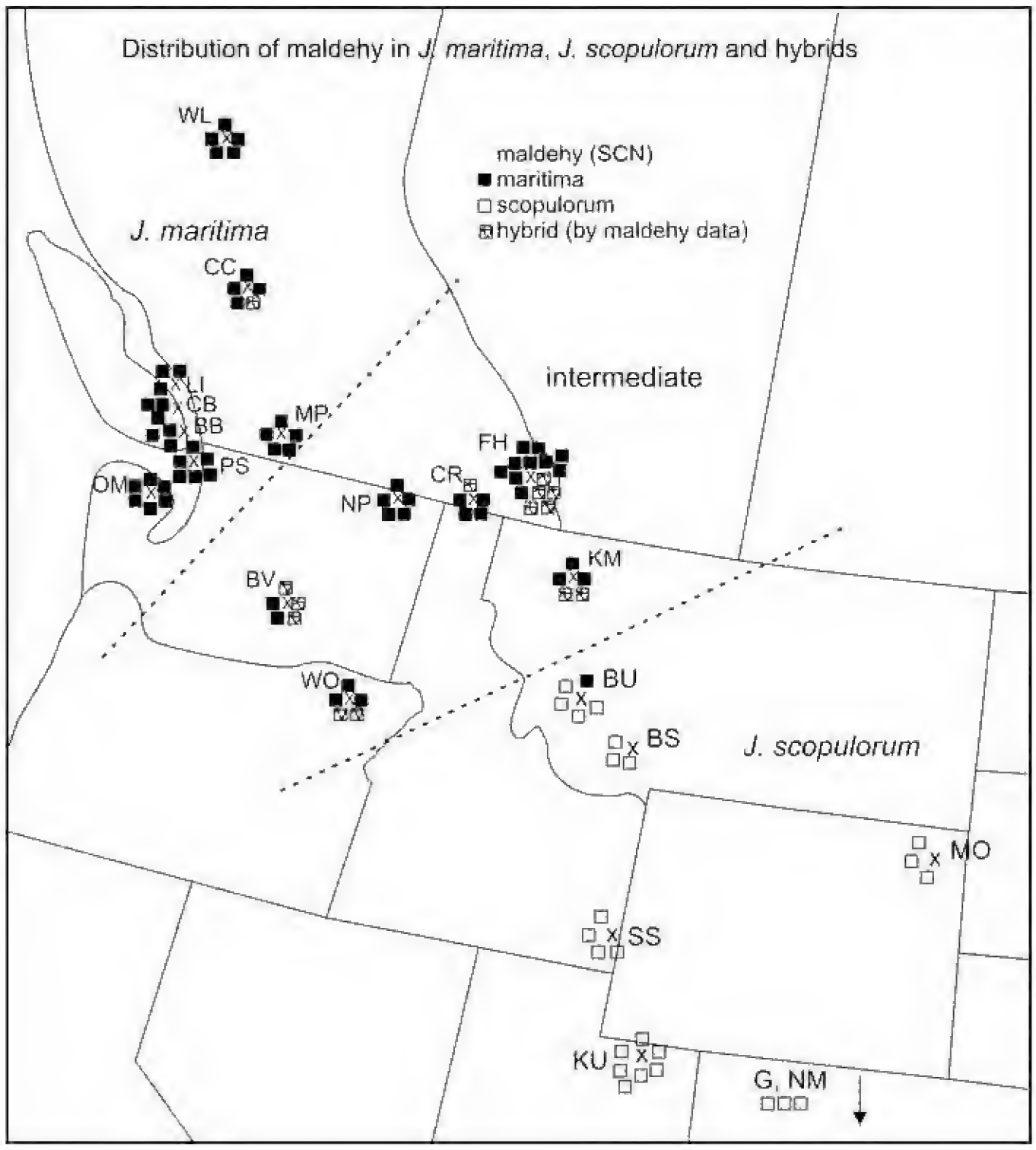


Figure 4. Distribution of *J. maritima, J. scopulorum* and intermediates as per the classification by their maldehy sequences.

Figure 5 shows combined mapping using all three gene classifications. The study area can be divided into roughly four zones:
1. typical *J. maritima*: Puget Sound, Vancouver Island, islands in the Strait of Georgia, and western BC;
2. intermediate trees: eastern WA, Wallowa, OR, se BC, and western MT;
3. trees introgressed from *J. maritima* into *J. scopulorum*: Montana and ne Wyoming;
4. mostly typical *J. scopulorum*: se ID, Utah and south in the Rocky Mtns.

The second zone contains two individuals (in the BB and CC populations) that were intermediate in maldehy, along with two individuals at Williams Lake (WL) that were intermediate in nrDNA (Fig. 4). No individuals that are pure in all three genes are present east of the dashed line (central BC and WA). Wallowa (WO) is the only eastern location in which individuals (2) contained *J. maritima* cpDNA (petN). Fairmont Hot Springs (FH) had the most hybrid individuals, as well as the only individual that was classified as an hybrid in both maldehy and nrDNA (Fig. 5).

nrDNA (ITS) differs by 5 bp between *J. maritima* and *J. scopulorum*. In the third zone several individuals had 2 or 3 nucleotides that were homozygous as *J. scopulorum*, and 3 or 2 that were heterozygous (*J. scopulorum* + *J. maritima*). This seems suggestive of a backcross or F_2 individual. It may be that some heterozygous trees are the result of previous hybridization, a relict from speciation, or the nrDNA may reflect concerted evolution in homogenizing individuals. Southeastern Idaho, Utah and New Mexico contain mostly pure *J. scopulorum.*

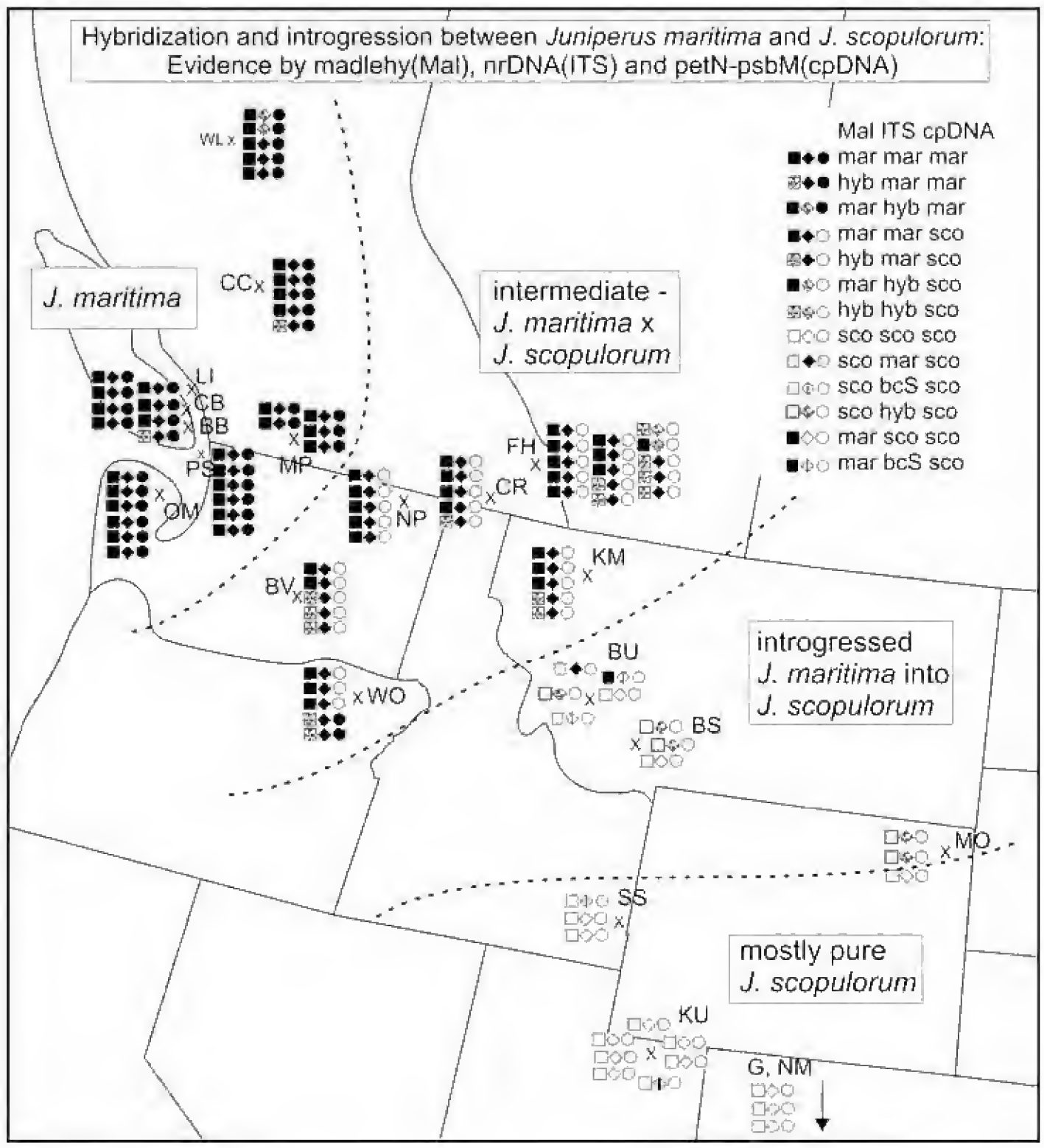


Figure5. Combined classification based on maldehy, nrDNA, and cpDNA sequences.

Inheritance of nrDNA

Chaing et al. (2001) found that in the artificial hybrids between *Begonia aptera* (pollen) and *B. formosana* (maternal), nrDNA was predominantly that of the maternal parent, *B. formosana* (diamonds, Fig. 6). This might explain the incongruence between the patterns for cpDNA (Fig. 2) and nrDNA (Fig. 3) in *Juniperus*, if maternal dominance is a factor in the inheritance of nrDNA.

Volkov, et al. (1999) reported that one of the parental nrDNAs was eliminated in the allopolyploid genome of cultivated tobacco. Fukuoka et al. (1994) found that the nrDNA in γ-ray irradiated tetraploid rice was homogenized in a short time.

Aguilar et al. (1999) made artificial hybrids between *Armeria villosa* ssp. *longiaristata* and *A. colorata*, then examined the inheritance of nrDNA in F_1 and F_2 generations. They found the expected additive pattern in polymorphisms for five of the six variable sites in F_1 plants. However, in the F_2 generation, there was a bias towards one parent (*A. colorata*). Backcrosses showed homogenization of five of the polymorphic sites to the recurrent parent.

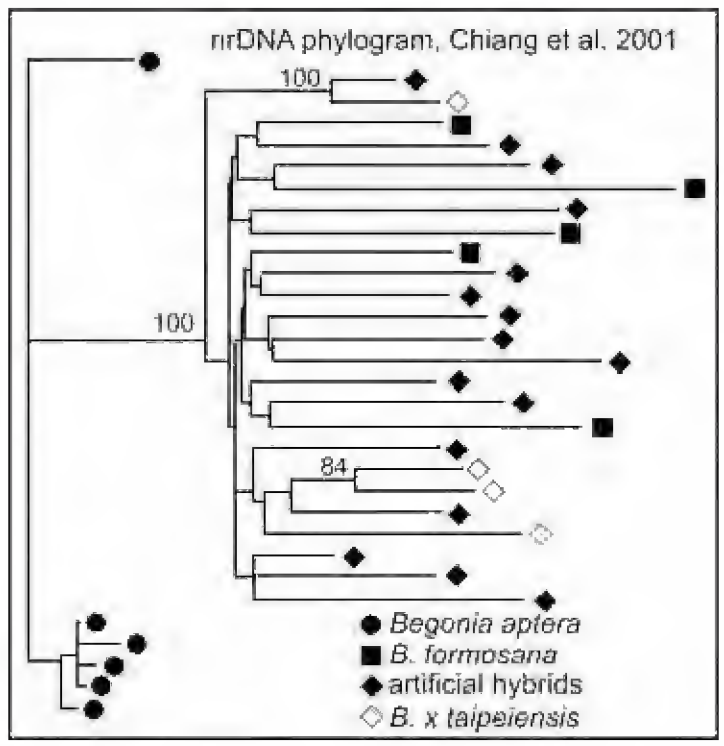


Figure 6. Phylogram based on nrDNA for *Begonia* and hybrids (adapted from Chiang, et al. 2001). Notice the grouping of the hybrids (triangles, nrDNA) with the maternal parent, *B. formosana* (shaded squares), rather than with the pollen (paternal) parent *(B. aptera*, shaded circles).

Okuyama et al. (2005) examined introgression in *Mitella* using nrDNA ITS and ETS, and cpDNA and found that cpDNA revealed the most introgression, ITS regions showed a moderate amount of introgression and the ETS region gave no evidence of introgression. They concluded that non-uniform concerted evolution between the ETS region and ITS regions may explain these different patterns of introgression.

Comparison with variation in leaf essential oils

Re-analysis of the terpene data, by removing CM (found to be *J. blancoi,* introgressed by *J. scopulorum*, Adams, 2011b, Adams, 2014) and adding *J. maritima* (MA, Vancouver Island, BC) shows MB oil (Manning Park, BC) similar to *J. maritima* oil (MA, Fig. 2). There appears to be a cline from MA (*J. maritima*) to Manning Park, to the DB, WO, KM, WB, TB group (Fig. 7).

Note also the differentiation of the Montana populations (BM, Butte, MT; BR, Bridger, MT; MM, Missouri River, n MT) from the uniform oils in the central Rocky Mtns. (Fig. 7). This is in close agreement with the zones based on all three gene regions (Fig. 5.)

However, it should be noted that although it seems intuitive that hybrids would have intermediate amounts of terpenes, Adams and Tsumura (2012) found that in *Cryptomeria japonica* hybrids, cis-thujopsene, widdrol and cedrol were inherited in Mendelian fashion with a second (dominant/recessive) gene involved. Several of the F_1 hybrids had oils very similar to the Haava parent's oil.

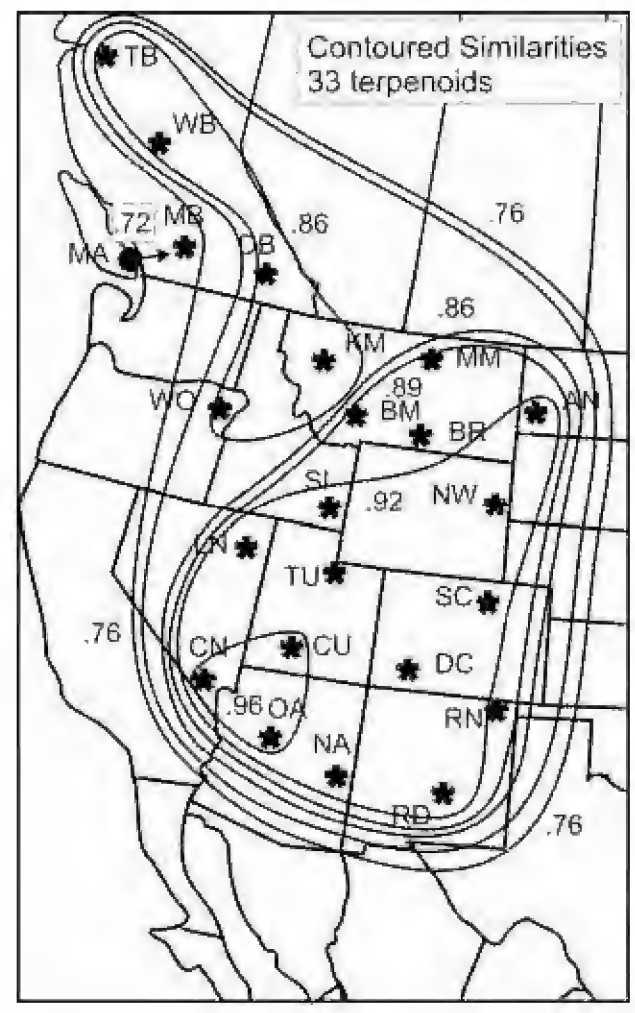


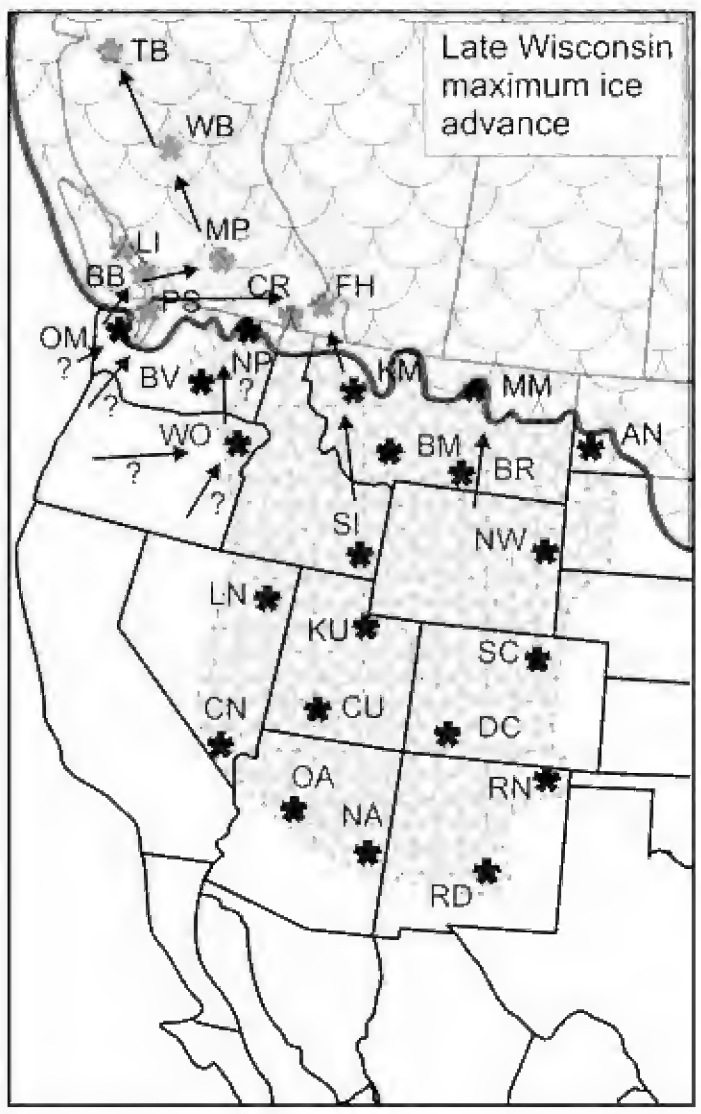
Fig. 7. Contour mapping of leaf oils. From Adams, 2011a.

In a study of the inheritance of the leaf terpenoids of *Pseudotsuga menziesii* var. *menziesii* x var. *glauca*, Adams and Stoehr (2013) found that cross *menziesii* 226 x *glauca* 267 produced 4 hybrids with oils very similar to the *glauca* parent and 6 F_1 hybrids with intermediate oils. In a second cross, of the 10 major terpenoids, 8 showed dominance values like one of the parents (Adams and Stoehr, 2013). Nine of

the terpenes were transgressive to both parents. So, it may not be unexpected that the contour mapping of terpenes (Fig. 7) show the oils of the putative hybrids to be more like one of the parents (*J. scopulorum*, Fig. 7).

Pleistocene Patterns

The late Wisconsin maximum ice advance is shown in figure 8 (based on Flint, 1971 and Crandell, 1971). All of the Canadian *J. maritima* and hybrid populations were glaciated. In addition, the Kalispell, MT (KM), Missouri River, MT (MM) and Amidon, ND (AN) populations were probably exterminated. Other populations (BM, BR and NW) were likely displaced by boreal forests and tundra (Flint, 1971; Porter, 1971). *Juniperus scopulorum* is a lower montane species. With the widespread lowering of vegetation zones, it likely moved to lower, drier habitats throughout most of the central Rocky Mountains. Adams (1983) reviewed the literature on packrat middens and pollen profiles. Wells (1970) and Martin and Harwell (1957) suggested that life zones descended 300 to 1100 m throughout the southwest and Great Basin from 13,500 to 10,000 ybp. The current separation of *J. scopulorum* and *J. virginiana* appears to have been bridged with the eastward expansion of *J. scopulorum* and the western expansion of *J. virginiana*. Trees of *J. scopulorum* are currently growing in ravines in northeastern New Mexico and western Oklahoma panhandle, while *J. virginiana* has now migrated westward into the Canadian River canyons in the Texas panhandle. The population of *J. scopulorum/virginiana* in Palo Duro Canyon resembles both species and is likely a relictual stand of hybrid origin (Adams, 1983).



With the retreat of the Wisconsin glacial ice, and the subsequent altithermal period 9000 to 5000 ybp (Wells, 1970), *Juniperus* expanded into the drying, higher elevation habitats that it occupies today. Figure 8 shows the proposed post-Pleistocene re-colonization of the northern portion of the ranges of *J. maritima* and *J. scopulorum.* The *J. maritima* BC populations could have been recolonized by seed from a Wallowa Mtns., OR refugium (WA, Fig. 8) and thence northward to the present day northern-most population at Telkwa, BC (TB). At Telkwa, *J. scopulorum* is found on dry, southeast facing slopes (ca. 45° - 60°). It seems likely that *J. maritima*, that grows along the seashore in western BC and Puget Sound, WA was re-colonized from a refugium south of the Olympic Mtns. or western WA/ Oregon.

Of course, the Wallowa population was likely displaced lower, and perhaps a bit to the south during the Wisconsin. The Amidon, ND (AN) population is similar to populations in the central Rocky Mountains and seems likely to have been derived by seed from the nearest *J. scopulorum* population (perhaps near Newcastle, WY, NW) or any of the scarp-land *J. scopulorum* populations to the south.

Figure 8. Putative re-colonization routes *J. maritima* and *J. scopulorum* following the Wisconsin (ice boundary based on Flint, 1971;Crandell, 1971).

ACKNOWLEDGEMENTS

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Table 1. Classification of 101 *Juniperus* individuals based on maldehy (SCN), nrDNA and petN-psbM (cpDNA). * newly reported in this paper. Sample numbers are Adams collection numbers.

Samples (trees)	maldehy	nrDNA	petN/psbM
10895 scopulorum, Kamas, UT	scop	scop	scop
10895 scopulorum, Kamas, UT	scop	scop	scop
10896 scopulorum, Kamas, UT	scop	scop	scop
10897 scopulorum, Kamas, UT	scop	scop	scop
10896 scopulorum, Kamas, UT	scop	scop	scop
10897 scopulorum, Kamas, UT	scop	scop	scop
13887 scopulorum, Kamas, UT*	scop	scop	scop
13888 scopulorum, Kamas, UT*	scop	**bc to scop**	scop
13889 scopulorum, Kamas, UT*	scop	scop	scop
13890 scopulorum, Kamas, UT*	scop	scop	scop
13891 scopulorum, Kamas, UT*	**marit**	scop	scop
10933 scopulorum, Glorietta, NM	scop	scop	scop
10934 scopulorum, Glorietta, NM	scop	scop	scop
10935 scopulorum, Glorietta, NM*	scop	scop	scop
7063 scopulorum, Soda Springs, ID*	scop	**bc to scop**	scop
7064 scopulorum, Soda Springs, ID*	scop	scop	scop
7065 scopulorum, Soda Springs, ID *	scop	scop	scop
7066 scopulorum, Soda Springs, ID*	scop	scop	scop
10876 scopulorum, Moorcroft, WY*	scop	scop	scop
10877 scopulorum, Moorcroft, WY*	scop	**hybrid**	scop
10878 scopulorum, Moorcroft, WY*	scop	**hybrid**	scop
10882 scopulorum, Big Sky MT*	scop	**hybrid**	scop
10883 scopulorum, Big Sky MT*	scop	**hybrid**	scop
10884 scopulorum, Big Sky MT*	scop	scop	scop
10885 scopulorum, Butte, MT*	scop	**hybrid**	scop
10886 scopulorum, Butte, MT*	scop	**marit**	scop
10887 scopulorum, Butte, MT*	scop	**bc to scop**	scop
10888 scopulorum, Butte, MT*	scop	scop	scop
10889 scopulorum, Butte, MT*	**marit**	**bc to scop**	scop
11056 maritima, Brentwood Bay, VI	marit	marit	marit
11057 maritima, Brentwood Bay, VI	marit	marit	marit
11058 maritima, Brentwood Bay, VI	marit	marit	marit
11061 maritima, Cowichan Bay, VI	marit	marit	marit
11062 maritima, Cowichan Bay, VI	marit	marit	marit
11063 maritima, Cowichan Bay, VI	**hybrid**	marit	marit
11999 maritima, Olympic Mtns., WA 912m,	marit	marit	marit
12000 maritima, Olympic Mtns., WA 912m,	marit	marit	marit
12001 maritima, Olympic Mtns., WA 912m,	marit	marit	marit
12002 maritima, Olympic Mtns., WA 1671m,	marit	marit	marit
12003 maritima, Olympic Mtns., WA 1671m,	marit	marit	marit
12004 maritima, Olympic Mtns., WA 1671m,	marit	marit	marit
11064 maritima, Yellow Point Lodge, VI	marit	marit	marit
11065 maritima, Lesqueti Island, BC	marit	marit	marit
11066 maritima, Lesqueti Island, BC	marit	marit	marit
11067 maritima, Friday Harbor, San Juan,WA	marit	marit	marit
11068 maritima, English Camp, San Juan, WA	marit	marit	marit

Table 1. Classification of 101 *Juniperus* individuals (contd.)

11075 maritima, sand dune, Whidbey Isl., WA	marit	marit	marit
11076 maritima, Fidalgo Isl. St. Pk, WA	marit	marit	marit
11077 maritima, Skagit Isl. WA, ca 360 yr old	marit	marit	marit
11078 maritima, Skagit Isl., WA	marit	marit	marit
13426 maritima, Manning Park, BC	marit	marit	marit
13427 maritima, Manning Park, BC	marit	marit	marit
13428 maritima, Manning Park, BC	marit	marit	marit
13429 maritima, Manning Park, BC	marit	marit	marit
13430 maritima, Manning Park, BC	marit	marit	marit
13431 Cache Ck, BC	marit	marit	marit
13432 Cache Ck, BC	marit	marit	marit
13433 Cache Ck, BC	**hybrid**	marit	marit
13434 Cache Ck, BC	marit	marit	marit
13435 Cache Ck, BC	marit	marit	marit
13436 Williams Lake, BC	marit	**hybrid**	marit
13437 Williams Lake, BC	marit	marit	marit
13438 Williams Lake, BC	marit	**hybrid**	marit
13439 Williams Lake, BC	marit	marit	marit
13440 Williams Lake, BC	marit	marit	marit
13421 Fairmont Hot Sprs, BC	**hybrid**	**hybrid**	**scop**
13422 Fairmont Hot Sprs, BC	marit	marit	scop
13423 Fairmont Hot Sprs, BC	marit	marit	scop
13424 Fairmont Hot Sprs, BC	marit	marit	scop
13425 Fairmont Hot Sprs, BC	marit	marit	scop
14001 Fairmont Hot Sprs, BC	marit	**hybrid**	scop
14002 Fairmont Hot Sprs, BC	marit	marit	scop
14003 Fairmont Hot Sprs, BC	marit	marit	scop
14004 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14005 Fairmont Hot Sprs, BC	marit	marit	scop
14006 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14007 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14008 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14009 Fairmont Hot Sprs, BC	**hybrid**	marit	scop
14010 Fairmont Hot Sprs, BC	marit	marit	scop
14026 Creston, BC	marit	marit	scop
14027 Creston, BC	marit	marit	scop
14028 Creston, BC	**hybrid**	marit	scop
14029 Creston, BC	marit	marit	scop
14030 Creston, BC	marit	marit	scop
14031 Northport, WA	marit	marit	scop
14032 Northport, WA	marit	marit	scop
14033 Northport, WA	marit	marit	scop
14034 Northport, WA	marit	marit	scop
14035 Northport, WA	marit	marit	scop
14036 Beverly, WA	**hybrid**	marit	scop
14037 Beverly, WA	marit	marit	scop
14038 Beverly, WA	marit	marit	scop
14039 Beverly, WA	**hybrid**	marit	scop
14040 Beverly, WA	**hybrid**	marit	scop
12995 Kalispell, MT	marit	marit	scop

Table 1. Classification of 101 *Juniperus* individuals (contd.)

12996 Kalispell, MT	marit	marit	scop
12997 Kalispell, MT	**hybrid**	marit	scop
12998 Kalispell, MT	**hybrid**	marit	scop
12999 Kalispell, MT	marit	marit	scop
11935 Wallowa Mtns, OR	**hybrid**	marit	scop
11936 Wallowa Mtns, OR	**hybrid**	marit	scop
11937 Wallowa Mtns, OR	marit	marit	scop
11938 Wallowa Mtns, OR	marit	marit	scop
11939 Wallowa Mtns, OR	marit	marit	scop

A morphometric analysis of *Arceuthobium campylopodum, A. laricis*, and *A. tsugense* (Viscaceae)

Robert L. Mathiasen
School of Forestry, Box 15018, Northern Arizona University, Flagstaff, Arizona 86011
Robert.Mathiasen@nau.edu

Shawn C. Kenaley
School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology Section,
Cornell University, Ithaca, New York 14853
sck26@cornell.edu

ABSTRACT

The classification of the dwarf mistletoes (*Arceuthobium* spp., Viscaceae) commonly parasitizing western hemlock (*Tsuga heterophylla*) and western larch (*Larix occidentalis*) in the northwestern United States and Canada has been one of the more difficult taxonomic problems associated with this important group of parasitic flowering plants. We collected new morphological measurements for the dwarf mistletoes parasitizing these commercially valuable conifers, *Arceuthobium tsugense* and *A. laricis*, respectively, from throughout most of their geographic ranges and used non-parametric univariate and multivariate statistical analyses to compare the morphological differences between them. In addition, because some investigators consider these taxa to be conspecific with, or subspecies of *A. campylopodum,* we included this dwarf mistletoe in our statistical analyses. Our analyses demonstrated that *A. tsugense* and *A. laricis* can be reliably segregated from each other, as well as from *A. campylopodum*, using plant heights, plant basal diameters, staminate spike widths, flower diameters, and fruit dimensions. Furthermore, their host affinities clearly distinguish them from each other. Therefore, we recommend they continue to be recognized as species. Morphological differences between these dwarf mistletoes are summarized and a key is provided for use in their identification. Published on-line www.phytologia.org *Phytologia 97(3): 200-218 (July 1, 2015).* ISSN 030319430.

KEY WORDS: *Arceuthobium* spp., Viscaceae , morphological characters, multivariate analyses, taxonomy, *Larix, Pinus*, *Tsuga.*

The dwarf mistletoes (*Arceuthobium* spp., Viscaceae) are among the most damaging parasites of commercially valuable conifers in the western United States and Canada (Hawksworth et al. 2002). Two of the most economically important species are western hemlock dwarf mistletoe (*Arceuthobium tsugense* (Rosendahl) G.N. Jones) and larch dwarf mistletoe (*A. laricis* (Piper) St. John) because they cause mortality and significant growth loss of severely infected western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and western larch (*Larix occidentalis* Nutt.), respectively (Hawksworth and Wiens 1996; Beatty et al. 1997; Hennon et al. 2001). Because these mistletoes are morphologically similar and flower and disperse seed at approximately the same time, their taxonomic classification has undergone many changes and is still under debate (Gill 1935; Hawksworth and Wiens 1972, 1996; Nickrent 2012).

The taxonomy of these dwarf mistletoe populations has become a topic of debate primarily because of recent molecular data (Nickrent et al. 2004). The molecular markers examined thus far indicate these taxa are very closely related to each other, as well as to *Arceuthobium campylopodum* Engelm., and therefore, their segregation from *A. campylopodum* as distinct species has been questioned (Nickrent et al. 2004; Baldwin et al. 2012). In addition, although the principal hosts of these dwarf mistletoes are distinct and represented by three different genera in the Pinaceae, these mistletoes also parasitize some of the same hosts. However, the susceptibility of their less commonly infected hosts varies considerably (Hawksworth and Wiens 1996; Mathiasen 1998; Mathiasen and Daugherty 2005).

In their first and since revised monograph of *Arceuthobium*, Hawksworth and Wiens (1972, 1996) recognized both *A. laricis* and *A. tsugense* as distinct species. Despite the morphological dissimilarities with *A. campylopodum* and the fact that *A. tsugense* does not parasitize *Pinus ponderosa* Douglas ex Lawson & C. Lawson or *P. jeffreyi* Grev. & Balf., the principal hosts of *A. campylopodum*, Nickrent et al. (2004) grouped *A. tsugense* under *A. campylopodum* based on molecular data. Furthermore, they grouped *A. laricis* under *A. campylopodum*, despite their morphological discontinuities and only occasional parasitism of *P. ponderosa* by *A. laricis*. The treatment for *Arceuthobium* by J. Kuijt in Baldwin et al. (2012) also grouped *A. tsugense* under *A. campylopodum*. These classifications have caused a good deal of uncertainty about the taxonomy of these economically and ecologically important dwarf mistletoes in the western United States and Canada, which has been further compounded by the recent recombination of *A. tsugense* and *A. laricis* as subspecies of *A. campylopodum* by Nickrent (2012).

We undertook this study because a detailed morphometric analysis comparing *Arceuthobium tsugense*, *A. laricis*, and *A. campylopodum* has not been completed. We collected a good deal of additional morphological data, far beyond the extent of that reported in Hawksworth and Wiens (1972, 1996) for *A. tsugense* (Wass and Mathiasen 2003), *A. campylopodum* (Mathiasen and Kenaley 2015), and *A. laricis* (reported here). This enabled us to compare the morphological characteristics of these three taxa using both a non-parametric univariate analysis and a more robust multivariate analysis using a relatively large data set. Our findings demonstrated that these species can readily be distinguished using many morphological characters and we have provided a key for use in their identification under field/laboratory conditions.

MATERIALS AND METHODS

The senior author and Mr. Ed Wass collected morphological data for 19 *Arceuthobium tsugense* populations distributed throughout most of its geographic range on *Tsuga heterophylla* in the United States and on Vancouver Island, British Columbia (Wass and Mathiasen 2003; Figure 1). We collected morphological data for *A. campylopodum* from 60 populations (30 each from *Pinus ponderosa* and *P. jeffreyi*) from throughout most of its geographic range (Mathiasen and Kenaley 2015; Figure 2). Lastly, we collected morphological data for *A. laricis* on *Larix occidentalis* from 32 populations distributed through most of its geographic range in the United States from 2011-2013 (Figure 3). Voucher specimens for *A. campylopodum* and *A. laricis* consisting of the mistletoe with host material were deposited at the Deaver Herbarium, Northern Arizona University, Flagstaff (ASC), or the University of Arizona Herbarium, Tucson (ARIZ). Voucher and specific population data, including GPS coordinates, have been archived electronically in SEINet (Southwest Environmental Information Network 2015): http://swbiodiversity.org/portal/index.php). Voucher specimens of *A. tsugense* were deposited at the Pacific Research Centre, Canadian Department of Forestry, Victoria, B. C., Canada (DAVFP).

For each mistletoe population, 10–20 male and 10–20 female infections (infected branches) were collected separately and the dominant plant (largest plant) from each infection was used for morphological measurements. The dwarf mistletoe plant characters measured were those used by Hawksworth and Wiens (1996) for the taxonomic classification of *Arceuthobium* taxa. The following morphological characters were measured: 1) height, basal diameter, third internode length and width, and color of male and female plants; 2) mature fruit length, width, and color; 3) seed length, width and color; 4) length and width of staminate spikes; 5) staminate flower diameters for 3- and 4-merous flowers; 6) length and width of staminate flower petals; and, 7) anther diameter and anther distance from the petal tip. Plant heights were measured to the nearest 0.1 cm and all other measurements were made to the nearest 0.1 mm.

Plants were usually measured within 12-h, but no later than 24-h after collection. Only plants that were still attached to their host's branch and were fully turgid were measured. Measurements were made using a digital caliper (Mitutoyo America Corp., Aurora, IL) and a 7X hand lens equipped with a micrometer (Bausch & Lomb, Bridgewater, NJ). The basal diameter of plants was measured at the point where the plant was attached to the host branch. The length and width of the third internode above the base of plants was included in our morphological analyses because these characters have been frequently reported for dwarf mistletoes and provide information on the relative size and thickness of male and female plants (Hawksworth and Wiens 1972, 1996; Mathiasen and Daugherty 2007, 2009a, 2009b, 2013; Mathiasen and Kenaley 2015). The length of the third internode was determined by measuring from the top of the second internode above the base of a plant to the top of the third internode, locations which are easily observed (see Figs. 2.1, 2.3, and 2.9 in Hawksworth and Wiens 1996). The width of the third internode was measured at its midpoint. Staminate spike and flower measurements were made during the peak of anthesis whereas fruit and seed measurements were made during the peak of seed dispersal. Sample sizes for most morphological characters measured varied among the three species sampled because of the number of populations sampled and the number of plants measured per population also varied. We purposely did not include samples of plants collected from hosts other than principal hosts for each dwarf mistletoe because there is some evidence that plants are smaller on less susceptible hosts (Mathiasen and Daugherty 2009b).

Statistical Analyses

We assessed whether values for morphological characters differed between and among species using Welch's *t* tests to accommodate unequal sample sizes and variances (Zimmerman 2004). Character differences between species were further assessed using the non-parametric Steel-Dwass, multiple comparison post hoc test ($\alpha = 0.05$). Standard and forward-stepwise quadratic discriminant function analyses (DFA) – powerful multivariate pattern-recognition methods – were also performed separately to determine whether female or male plants of *Arceuthobium campylopodum*, *A. laricis*, and *A. tsugense* can be delimited to species using only morphological characters (Quinn and Keough 2002). Discriminant function analyses classification compared actual species membership defined a priori via field diagnosis to predicted species memberships according to only continuous female (n= eight characters) or male morphologies (n= 10 characters). Separate DFAs for female and male plants were executed using equal prior probabilities for each species (25%) rather than proportional to their occurrences in the data set(s) as previous molecular phylogenetic analyses failed to resolve these taxa to separate species (Nickrent et al. 2004). Standardized correlation coefficients for morphological characters of female and male plants were also calculated to assess the contribution of each character to the discriminant function; thereby, providing the principal morphologies separating the dwarf mistletoes. Likewise, stepwise DFA was utilized to examine systematically the smallest number of morphological characteristics, female or male, resulting in the highest precision in species classification (%, actual/predicted). To do this, stepwise models were executed using equal prior probabilities and the sequential addition of morphological characters most-to-least correlated to the discriminant function. The full-model DFA was validated by resampling separately the original (complete) data set for female and male plants; selecting at random 50 complete records per species and re-performing the DFA using all morphological characteristics simultaneously. Non-parametric tests and DFAs were computed in JMP Pro 10 (SAS Institute, Cary, North Carolina, USA).

RESULTS

The mean heights of female and male plants of *Arceuthobium campylopodum* were significantly greater than *A. tsugense* and *A. laricis* and those of *A. tsugense* were significantly greater than those of *A. laricis* (Table 1). The mean basal diameters of female and male plants were also significantly different for all three taxa. The mean lengths of the third internodes of female plants was significantly different for all

three species, but the mean lengths of the third internode of male plants was not significantly different between *A. campylopodum* and *A. tsugense*. The third intermodal length for male plants of the latter taxa (*A. campylopodum* and *A. tsugense*), however, was significantly greater when compared to *A. laricis*. Although the mean widths of the third internodes of both male and female plants were significantly different between each species, the mean widths were similar for *A. tsugense* and *A. laricis* (1.6 and 1.7 mm, respectively) (Table 1). But the mean width of the third internode for *A. campylopodum* was greater (2.5 mm) than the latter two taxa. The staminate spikes of *A. campylopodum* were significantly longer on average than the other species, but those of *A. tsugense* and *A. laricis* were not significantly different (Table 1). The mean width of staminate spikes was significantly different between all three taxa, with those of *A. campylopodum* the largest and *A. tsugense* had the most slender staminate spikes.

The mean diameter of 3-merous flowers was significantly smaller for *Arceuthobium laricis* and similar for the other two species (Table 1). The mean diameter of 4-merous flowers was similar for *A. tsugense* and *A. laricis* and largest for *A. campylopodum*, but the mean diameters were all significantly different. The mean lengths of petals only varied by 0.1 mm between all three species, but the means were significantly different. The mean width of petals was widest for *A. campylopodum* and was significantly different than the mean petal width for the other two species, which were both 1.2 mm. Mean anther diameters only varied from 0.5-0.7 mm, but were significantly different among species. The mean distance of anthers from the tips of petals was greatest for *A. campylopodum* (0.6 mm) and it was significantly different from the means for the other taxa which were 0.5 mm.

Mean fruit length was larger for *Arceuthobium campylopodum* (5.4 mm) and although the mean fruit lengths were similar for *A. tsugense* and *A. laricis* (4.4 and 4.3 mm, respectively), they were significantly different (Table 1). The mean width of fruits was also larger for *A. campylopodum* (3.7 mm) while the means for the other species (2.9 and 3.0 mm) were not significantly different. The mean seed length was significantly different between all three species, and the seeds of *A. campylopodum* were larger (3.5 mm) as was the mean width of its seeds (1.5 mm). Although the mean width of the seeds for *A. tsugense* and *A. laricis* were similar (1.1 and 1.2 mm, respectively), they were significantly different. The principal characters that can be used to differentiate between *A. campylopodum*, *A. laricis*, and *A. tsugense* are summarized in Table 2.

Plant color is not usually an informative character for distinguishing between dwarf mistletoes. However, the color of plants of *A. laricis* was distinctly different from those of the other two species, being green-brown to reddish or almost purple. Plants of *A. tsugense* and *A. campylopodum* were predominantly yellow-green, green, or yellow-brown.

Separate quadratic discriminant function analyses (DFA) utilizing complete data for eight female and 10 male morphological characters (i.e., full-models) resulted in an overall correct classification rate of 94.3% (783/830) and 96.2% (982/1021), respectively (Table 3), and hence, readily separated *Arceuthobium campylopodum*, *A. laricis*, and *A. tsugense* according to species membership. Multivariate analysis of variance (MANOVA) indicated that the classification to predicted species of female (Wilks' λ= 0.1398, Approximant $F_{16,1640}$= 171.6, $P < 0.0001$; Pillai's Trace= 1.08, Approximant $F_{16, 1642}$= 120.2, $P < 0.0001$) and male plants (Wilks' λ= 0.1347, Approximant $F_{20, 2018}$= 174.0, $P < 0.0001$; Pillai's Trace= 1.17, Approximant $F_{20, 2020}$= 142.1, $P < 0.0001$) was significantly different from random. Means and associated 95% confidence intervals for morphological characters of female and male plants across predicted species according to full-model DFA are presented in Table 4.

Using the complete dataset and eight female characters, DFA correctly classified plants (predicted/actual) of *Arceuthobium campylopodum*, *A. laricis*, *A. tsugense* to species 97.7% (469/480), 92.0% (138/150), and 88.0% (176/200) of the time (Table 3), respectively, with the first discriminant function (canonical) explaining 91.9% of the total variation (Table 5). The latter taxon, *A. tsugense*, was

most often misclassified as *A. laricis* (8.5%; 17/150) and rarely to *A. campylopodum* (3.5%; 7/150) (Table 3). However, female plants diagnosed upon field identification as *A. laricis* were never delimited morpholgically to *A. campylopodum*, and vise versa, no specimens of *A. campylopodum* determined a priori were classified to *A. laricis*. Standardized correlation coefficients for DFA using the complete and resampled datasets, indicating the relative importance of individual female morphological characters in defining the discriminant functions, are listed in Table 6. Third internode length and width, seed length, and fruit length were most strongly correlated with the first and second canonical, and hence, contributed most to defining species membership when only female plants were considered. Using these four, female characters alone, the overall correct classification via DFA was 92.3% (766/830) with *A. campylopodum*, *A. laricis*, and *A. tsugense* classified correctly 97.1% (466/480), 92.0% (138/150), 81.0% (162/200) of the time, respectively (Table 3). The sequential addition of female morphologic characters (predicator variables) beyond those most correlated to the discriminant functions (female, steps 5-8, Table 3) only increased the correct classification rate for *A. tsugense* by seven percent to the maximum 88% and contributed little to further differentiate *A. tsugense* from *A. campylopodum* or *A. laricis* (Tables 3 and 6). Although female plants of *A. tsugense* were misclassified ≤ 8.5% of the time when eight morphological characters were considered (full-model), the multivariate mean and 95% confidence ellipse for this taxon – as well as *A. campylopodum* or *A. laricis* – did not intersect in multivariate space with those of the other taxa when analyses were executed with either the complete or resampled dataset (Figure 4).

Predicting species membership via DFA for male plants of *Arceuthobium laricis* and *A. tsugense,* was improved in comparison to DFA results for female plants, fruits, and seeds as an overall correct classification rate ≥ 97.3% was attained for both taxa using complete data and all 10 male characters (Table 3). The first and second discriminant functions described 84.7% and 15.3% of the variation among male plants (Table 5). Full-model DFA on male plants using complete or resampled data clearly differentiated all three taxa (Table 3; Figure 4). Analyses with complete data rarely misclassified *A. tsugense* as *A. laricis* (0.8%; 2/261) or *A. campylopodum* (1.9%; 5/261), and *A. laricis* was ascribed to *A. campylopodum* only 2.5% (4/160) of the time. Moreover, as demonstrated previously by DFAs of female plants, male plants identified in the field as *A. campylopodum* were also consistently and correctly classified as *A. campylopodum* (86.8-95.3%; 521-572/600) and were readily distinguishable from *A. laricis* and *A. tsugense* when considering as few as four morphological characters. Interestingly, DFA of male plants utilizing only two or three characters resulted in an overall correct classification rate of 92.3% (942/1021) or 91.6% (935/1021), respectively. Male morphology contributing greatest to the discrimination among *A. campylopodum*, *A. laricis*, and *A. tsugense* included third internode width, staminate spike width, anther diameter, third internode length, and anther distance from tip (Table 6), whereas, plant height, basal diameter, staminate spike length, and petal length and width contributed least to determining species membership. Male plant characters contributing most to the species discrimination were consistent between the complete and resampled datasets (Table 6), and the position of multivariate means across the three taxa in ordination space were maintained when comparing DFAs executed with either dataset (Figure 4).

DISCUSSION

Classifying *Arceuthobium tsugense* and *A. laricis* as conspecific or even subspecies of *A. campylopodum* is not supported by our analyses of the morphological characters we measured for these taxa. All three of these species can be reliably identified by differences in their plant heights, basal diameters, and width of their staminate spikes (Table 2). The mean widths of the third internode and the fruits and seeds of *A. campylopodum* were larger than those produced by the other taxa, which were about the same size. Characters that distinguished *A. laricis* from the other two species were its smaller male and female plants, its shorter third internode lengths, and smaller 3-merous flowers. Furthermore, although plant color is a qualitative character, *A. laricis* can be distinguished using plant color alone; it is typically green-brown, red, or purple.

The host range of *Arceuthobium tsugense* is distinct from both *A. campylopodum* and *A. laricis*; its principal host being *Tsuga heterophylla* and it does not parasitize *Pinus ponderosa* and *P. jeffreyi*, or *Larix occidentalis*, the principal hosts of *A. campylopodum* and *A. laricis*, respectively. Furthermore, *T. heterophylla* is very rarely infected by *A. laricis* (Hawksworth 1987) and is probably immune to infection by *A. campylopodum*. Because *T. heterophylla* and *P. ponderosa* are seldom sympatric, the latter host-mistletoe combination has not yet been verified (Hawksworth and Wiens 1996; Mathiasen and Daugherty 2005). Although *A. laricis* has been reported to parasitize *P. ponderosa*, it only occasionally infects this host in stands where it is also parasitizing *L. occidentalis* (Hawksworth and Wiens 1996; Mathiasen 1998). Likewise, although *Tsuga mertensiana* is considered a secondary host for *A. laricis* (Mathiasen 1998), this host is only occasionally parasitized by *A. tsugense* (Shaw 1982; Mathiasen and Daugherty 2005). While the susceptibility of *T. mertensiana* to *A. campylopodum* remains unknown, it is probably not susceptible.

Although *Abies amabilis* has been artificially inoculated successfully with *Arceuthobium laricis* (Smith and Wass 1972), this host is only rarely infected by *A. laricis* in Washington (Mathiasen et al. 1995). Therefore, although some of the same hosts are infected by both *A. laricis* and *A. tsugense*, the susceptibility of the hosts they have in common varies considerably (Table 2). None of the dwarf mistletoes share a principal host in common and the principal host of *A. tsugense* (*Tsuga heterophylla*) has only been reported to be infected by *A. laricis* from one location in Washington (Hawksworth 1987). Furthermore, *A. campylopodum* has never been reported naturally parasitizing a species of *Tsuga* or *Larix*. The host ranges of these dwarf mistletoes is further evidence they are genetically distinct and should be given separate taxonomic status. These three dwarf mistletoes clearly have very different host ranges and hence, their pathological significance in forests of the northwestern United States and Canada is also distinct. Furthermore, because their host ranges are so remarkably different, any efforts to manage populations of these parasites to mitigate their potential for causing growth loss and mortality of severely infected trees, must consider these differences (Hawksworth and Wiens 1996).

Some treatments of *Arceuthobium tsugense* now segregate this taxon into four subspecies based on morphology, phenology, geographic distribution, and host specialization (Hawksworth et al. 1992; Hawksworth and Wiens 1996; Wass and Mathiasen 2003; Mathiasen and Daugherty 2007). Because the morphological and host affinities of the subspecies have already been outlined in this literature, we only compared the morphological characteristics of *A. tsugense* subsp. *tsugense* with those of *A. laricis* and *A. campylopodum* in this study. It should be noted that although *Tsuga mertensiana* is a secondary host of *A. laricis* and a principal host of *A. tsugense* (Rosendahl) G. N. Jones subsp. *mertensianae* Hawksw., Wiens, & Nickrent, these dwarf mistletoes are morphologically distinct and geographically isolated from each other (Hawksworth et al. 1992; Hawksworth and Wiens 1996). Although, *A. tsugense* subsp. *mertensianae* has been reported from central and northern Oregon, Washington, and southern British Columbia (Hawksworth et al. 1992; Hawksworth and Wiens 1996), it is now known that these populations are based on parasitism of *T. mertensiana* by *A. tsugense* subsp. *amabilae* in central and northern Oregon (Mathiasen and Daugherty 2007) and by *A. laricis* in Washington and British Columbia (Mathiasen unpublished data).

Nickrent (2012) proposed that taxa within section *Campylopoda* Hawksw. & Wiens, series *Campylopoda* conform to the concept of ecotypes. He based his conclusion on the fact that because seeds of *Arceuthobium campylopodum* collected from one host tree may not survive on another host, species in series *Campylopoda* would best be represented as ecotypes since the most important environmental component affecting dwarf mistletoe survival and reproduction was the host tree. He contended that host specificity was not common for dwarf mistletoes because several of the subspecies he recognized have four principal hosts and parasitism of occasional and rare hosts overlaps between species. In reality, only *A. cyanocarpum* (sensu stricto) has four principal hosts, all closely related white pines (Hawksworth and Wiens 1996). Nickrent's (2012) summary of principal hosts for his subspecies of *A. campylopodum* was

based on his grouping of several taxa we recognize as subspecies of *A. abietinum*, *A. microcarpum*, and *A. tsugense*. This greatly skewed his principal host totals and misrepresented the actual host specificity of these three species. Following our interpretation of the subspecific classification of these species, none of them has more than two principal hosts. In addition, Nickrent's discussion of host specificity did not consider the severity of infection observed for secondary, occasional, or rare hosts or the fact that in many cases few mistletoes shoots are produced on occasional and particularly rare hosts; thereby indicating that many of the cases of dwarf mistletoe infection on less susceptible hosts are what Hawksworth and Wiens (1996) termed "incompatible." Furthermore, Nickrent (2012) did not discuss the importance of immune hosts and why this must also be considered when assessing the significance of host affinities and the taxonomic classification of dwarf mistletoes.

When dwarf mistletoe-host relationships are compared to other mistletoes, dwarf mistletoes are relatively host specific because many species, including the species studied here, only infect one or two species as principal hosts. Host preferences are indications of genetic differences between dwarf mistletoes and the greater the differences in the host's taxonomic relationships for principal as well as immune hosts, the greater the genetic differences between the dwarf mistletoes that preferentially infect or will not infect hosts which are phylogenetically closely related or unrelated (Hawksworth 1990). Furthermore, other investigators have demonstrated the large differences in the host affinities of the dwarf mistletoes studied here (Hawksworth and Wiens 1972, 1996; Mathiasen 1994, 1998; Mathiasen and Daugherty 2005). Obviously, we disagree with Nickrent's (2012) assertion that dwarf mistletoes are not host specific parasitic plants simply because they sometimes have more than one principal host and infect other species to varying degrees. In contrast, we consider dwarf mistletoe-host relationships to be critical in their taxonomic classification. Accordingly, we support the classification of dwarf mistletoe populations as species that primarily parasitize unrelated host genera such as *Pinus*, *Tsuga*, and *Larix* as principal hosts.

Nickrent (2012) also argued that since the range in plant dimensions (height, basal diameter, third internode dimensions) overlapped, had similar ITS and chloroplast DNA sequences, and that several species were sympatric, all of the members of series *Campylopoda* should be treated as subspecies of *A. campylopodum*. It is important to note that Nickrent (2012) concluded that there were adequate morphological and physiological differences between the species in series *Campylopoda* to warrant their at least having subspecific status under *A. campylopodum*. This was a major modification of his earlier recommendation that all the taxa in series *Campylopoda*, except *A. blumeri* A. Nelson, were conspecific (Nickrent et al. 2004). Our results have clearly demonstrated using statistical analyses of the many morphological characters we examined that *A. tsugense* and *A. laricis* are morphologically distinct from each other and even more so from *A. campylopodum*.

Considering the morphological data presented herein for *Arceuthobium campylopodum*, *A. tsugense*, and *A. laricis* and evident discontinuities in the host affinities among these taxa, we maintain that it is more consistent with other specific classifications of dwarf mistletoe populations to continue classifying these taxa as species. Recognition of the host affinities developed by dwarf mistletoes is critical in their classification because we consider differences in host preference(s) to reflect corresponding and underlying genetic differentiation between dwarf mistletoes. Our morphological analyses demonstrated that these species are readily separated using several characters (Table 2; Figure 4) and field observations of their host affinities have also demonstrated that they are genetically distinct in that they parasitize taxonomically distinct members of the Pinaceae as their principal hosts and only infect closely related conifers to a lesser degree.

KEY FOR IDENTIFICATION OF *ARCEUTHOBIUM LARICIS*, *A. TSUGENSE*, AND *A. CAMPYLOPODUM*

1. Plants green-brown, reddish, or purple, plants heights usually less than 6 cm; basal diameters usually less than 2.5 mm; 3-merous flowers less than 3 mm in diameter; primarily parasitic on *Larix occidentalis*, *Pinus contorta*, or *Tsuga mertensiana* ..... *Arceuthobium laricis*

1'. Plants yellow-brown, yellow-green, green, or brown at their base; plant heights usually greater than 6 cm; basal diameters usually greater than 2.5 mm; 3-merous flowers greater than 3 mm in diameter; primarily parasitic on *Pinus ponderosa*, *P. jeffreyi*, or *Tsuga heterophylla*..... 2

2. Plant heights usually less than 9 cm; basal diameters usually less than 3 mm; width of third internode usually less than 2 mm; staminate spike width usually less than 2 mm; mature fruits less than 5 mm long and 3 mm wide; seeds about 2.5 x 1.1 mm; primarily parasitic on *Tsuga heterophylla*..... *Arceuthobium tsugense*

2'. Plant heights usually greater than 9 cm; basal diameters usually greater than 3 mm; width of third internode usually greater than 2 mm; staminate spike width usually greater than 2 mm; mature fruits typically greater than 5 mm long and 3 mm wide; seeds about 3.5 x 1.5 mm; primarily parasitic on *Pinus ponderosa* and *P . jeffreyi*; also common on *P. attenuata* and *P. coulteri* ..... *Arceuthobium campylopodum*

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Table 1. Morphological measurements for *Arceuthobium campylopodum*, *A. tsugense*, and *A. laricis*. Data are listed as **mean**, (SD) [n]. Means followed by different superscripted letters in the same row were significantly different using Welch's *t* tests and the nonparametric Steel-Dwass, multiple comparison post hoc test ($\alpha = 0.05$). Lower case letters in the brackets designate sample sizes already listed in the same column. Plant heights are in cm and all other measurements in mm.

Character	***Arceuthobium campylopodum***	***Arceuthobium laricis***	***Arceuthobium tsugense***
Plant height			
Female	**10.4**^{a} (2.7) [600a]	**5.3**^{b} (1.3) [160a]	**7.8**^{c} (2.2) [270a]
Male	**9.7**^{a} (3.0) [a]	**4.7**^{b} (1.3) [a]	**8.0**^{c} (2.0) [265b]
Basal diameter			
Female	**3.4**^{a} (0.7) [a]	**2.4**^{b} (0.5) [a]	**2.7**^{c} (0.8) [a]
Male	**3.2**^{a} (0.6) [a]	**2.1**^{b} (0.5) [a]	**2.6**^{c} (0.5) [b]
Third internode length			
Female	**13.0**^{a} (3.1) [a]	**8.5**^{b} (2.0) [a]	**12.3**^{c} (3.2) [a]
Male	**12.0**^{a} (3.3) [a]	**7.5**^{b} (1.9) [a]	**11.8**^{a} (3.3) [b]
Third internode width			
Female	**2.5**^{a} (0.4) [a]	**1.7**^{b} (0.2) [a]	**1.6**^{c} (0.4) [a]
Male	**2.5**^{a} (0.4) [a]	**1.7**^{b} (0.2) [a]	**1.6**^{c} (0.3) [b]
Staminate spike length	**12.7**^{a} (4.8) [760b]	**10.1**^{b} (3.1) [240b]	**10.8**^{b} (2.7)[260c]
Staminate spike width	**3.0**^{a} (0.3) [b]	**2.6**^{b} (0.2) [b]	**1.6**^{c} (0.1) [c]
Flower diameter			
3-merous	**3.1**^{a} (0.4) [400]	**2.7**^{b} (0.3) [120c]	**3.2**^{a} (0.5) [115d]
4-merous	**4.2**^{a} (0.5) [360]	**3.7**^{b} (0.3) [c]	**3.8**^{c} (0.5) [d]
Petal lobe length	**1.6**^{a} (0.2) [b]	**1.4**^{b} (0.2) [b]	**1.5**^{c} (0.2) [c]
Petal lobe width	**1.4**^{a} (0.2) [b]	**1.2**^{b} (0.2) [b]	**1.2**^{c} (0.2) [c]
Anther diameter	**0.6**^{a} (0.1) [b]	**0.5**^{b} (0.1) [b]	**0.7**^{c} (0.1) [c]
Anther distance from tip	**0.6**^{a} (0.1) [b]	**0.5**^{b} (0.1) [b]	**0.5**^{b} (0.2) [c]
Fruit length	**5.4**^{a} (0.5) [480c]	**4.3**^{b} (0.4) [150d]	**4.4**^{c} (0.4) [210e]
Fruit Width	**3.7**^{a} (0.4) [c]	**3.0**^{b} (0.3) [d]	**2.9**^{b} (0.2) [e]
Seed length	**3.5**^{a} (0.4) [c]	**2.4**^{b} (0.3) [d]	**2.6**^{c} (0.3) [200f]
Seed width	**1.5**^{a} (0.2) [c]	**1.2**^{b} (0.1) [d]	**1.1**^{c} (0.1) [f]

Table 2. Summary of the principal characters separating *Arceuthobium campylopodum*, *A. tsugense*, and *A. laricis*. Data for morphological characters are means; plant heights in cm and all other measurements in mm. Numbers in bold represent key morphological or phenological differences between the taxa. Host susceptibility classification based on information in Hawksworth (1987), Hawksworth and Wiens (1996), Mathiasen (1998), and Mathiasen and Daugherty (2005, 2007).

Character		***Arceuthobium campylopodum***	***Arceuthobium laricis***	***Arceuthobium tsugense***
Plant height				
	Female	**10.4**	**5.3**	**8**
	Male	**9.7**	**4.7**	**7.8**
Plant color		Yellow-green, green, yellow-brown	Brown-green, red, purple	Yellow-green, green, yellow-brown
Basal diameter				
	Female	**3.4**	**2.4**	**2.7**
	Male	**3.2**	**2.1**	**2.6**
Third internode width				
	Female	**2.5**	1.7	1.6
	Male	**2.5**	1.7	1.6
Staminate spike width		**3.1**	**2.6**	**1.6**
Flower diameter				
	3-merous	3.1	**2.7**	3.2
	4-merous	**4.2**	3.7	3.8
Fruit length		**5.4**	4.3	4.4
Fruit width		**3.7**	3	2.9
Sympatry among taxa		*A. laricis*	*A. campylopodum*	Not sympatric
Host Susceptibility				
	Principal	*Pinus jeffreyi*; *P. ponderosa*	*Larix occidentalis*	*Tsuga heterophylla*
	Secondary	*P. attenuata*; *P. coulteri*	*Tsuga mertensiana*; *P. contorta var. latifolia*	None
	Occasional	*P. contorta* var. *murrayana*, var. *latifolia; P. sabiniana*	*Abies lasiocarpa*; *Pinus ponderosa*	*Abies amabilis; A. grandis*; *A.procera; Pinus contorta var.latifolia; Tsuga mertensiana*
	Rare	*P. lambertiana*	*Abies grandis*; *Picea engelmannii; Pinus albicaulis; P. monticola; Tsuga heterophylla*	*Picea engelmannii*; *P. sitchensis; Pinus monticola; Pseudotsuga menziesii*
	Immune	*Abies grandis*		*P. contorta* var. *murrayana*

Table 3. Predicted taxonomic membership according to forward, stepwise discriminant function analyses (DFA) for the morphological classification of female (n= 8 characters) and male plants (n= 10 characters) using complete data. Correct classification (%, predicted/actual) per species-taxon membership combination greater than 90% are ar in **boldface**. Anther diameter (AD); anther distance from tip (ADT); basal diameter (BA); fruit length (FL); fruit width (FW); petal length (PL); petal width (PW); plant height (PH); staminate spike length (SSL); staminate spike width (SSW); third internode length (TIL); and, third internode width (TIW). Sample size (n; female, male plants): *Arceuthobium campylopodum* (480, 600), *A. laricis* (150, 160), and *A. tsugense* (200, 261).

Stepwise DFA (Step [Character])	**Species by predicted taxonomic membership (%, predicted/actual)**									
	Total	*A. campylopodum* (*Ac*)			*A. laricis* (*Al*)			*A. tsugense* (*At*)		
		Ac	*Al*	*At*	*Ac*	*Al*	*At*	*Ac*	*Al*	*At*
Female (n = 830 total plants)										
1. [**SL**]	71.1	80	2.3	17.7	2.7	74.7	22.7	6.5	46.5	47
2. [SL], [**TIW**]	84.3	94.8	0.4	4.8	1.3	78	20.7	7	29	64
3. [SL], [TIW], [**TIL**]	90.7	**95.6**	1	3.3	1.3	**90.0**	8.7	7	13.5	79.5
4. [SL], [TIW], [TIL], [**FL**]	92.3	**97.1**	0.8	2.1	0.7	**92.6**	6.7	5.5	13.5	81
5. [SL], [TIW], [TIL], [FL], [**SL**]	92.9	**97.1**	0.6	2.3	1.3	**91.3**	7.3	5	11	84
6. [SL], [TIW], [TIL], [FL], [SL], [**PH**]	94.2	**97.7**	0	2.3	0	**92.7**	7.3	4.5	8.5	87
7. [SL], [TIW], [TIL], [FL], [SW], [PH], [**FW**]	94.3	**97.7**	0	2.3	0	**93.3**	6.7	4	9	87
8. [SL], [TIW], [TIL], [FL], [SW], [PH], [FW], **[BD]**	94.3	**97.7**	0	2.3	**0**	**92.0**	8	3.5	8.5	88
Male (n = 1021 total plants)										
1. [**TIW**]	74.4	86.8	13	0.2	5.6	85.0	9.4	5.7	54.8	39.5
2. [TIW], [**SSW**]	92.3	**92.2**	6.8	1	3.8	**94.4**	1.9	4.6	4.2	**91.2**
3. [TIW], [SSW], [**AD**]	91.6	**91.3**	7	1.7	3.8	**91.3**	5	4.2	3.4	**92.3**
4. [TIW], [SSW], [AD], [**TIL**]	94.1	**93.2**	5.2	1.7	3.1	**96.3**	0.6	3.1	1.9	**95.0**
5. [TIW], [SSW], [AD], [TIL], [**ADT**]	94.9	**93.7**	5.3	1	3.1	**96.3**	0.6	1.5	1.5	**96.9**
6. [TIW], [SSW], [AD], [TIL], [ADT], [**PH**]	95.7	**94.3**	4.3	1.3	1.3	**97.5**	1.3	1.5	0.8	**97.7**
7. [TIW], [SSW], [AD], [TIL], [ADT], [PL], [**BA**]	95.6	**94.5**	4.3	0.8	1.9	**96.9**	1.3	1.9	0.8	**97.3**
8. [TIW], [SSW], [AD], [TIL], [ADT], [PL], [BA], [**SSL**]	96.2	**95.3**	3.7	1	1.3	**97.5**	1.3	1.9	0.8	**97.3**
9. [TIW], [SSW], [AD], [TIL], [ADT], [PL], [BA], [SSL], [**PW**]	95.9	**94.5**	4.3	1.2	1.9	**98.1**	0	1.5	0.8	**97.7**
10. [TIW], [SSW], [AD], [TIL], [ADT], [PL], [BA], [SSL], [PW], [**PL**]	96.2	**95.3**	3.7	1	2.5	**97.5**	0	1.9	0.8	**97.3**

Table 4. Quadratic discriminant function analyses (DFA) of male and female plants using complete data for *Arceuthobium campylopodum*, *A. laricis*, and *A. tusgense*. Comparison of morphological characters (means) according to predicted classification to species. Ninety-five percent confidence intervals (±) were computed for comparison of mean differences. Mean plant heights in cm and all other measurements in mm.

Character		***A. campylopodum***	***A. laricis***	***A. tsugense***
Plant height				
	Female	10.3 (±0.24)	5.6 (±0.21)	8.1 (±0.31)
	Male	9.8 (±0.24)	4.8 (±0.19)	7.8 (±0.27)
Basal diameter				
	Female	3.4 (±0.06)	2.4 (±0.07)	2.5 (±0.10)
	Male	3.2 (±0.03)	2.2 (±0.07)	2.6 (±0.08)
Third internode length				
	Female	13.1 (±0.27)	8.7 (±0.30)	12.9 (±0.49)
	Male	12.0 (±0.27)	7.7 (±0.28)	11.8 (±0.39)
Third internode width				
	Female	2.5 (±0.03)	1.7 (±0.04)	1.5 (±0.05)
	Male	2.5 (±0.03)	1.7 (±0.03)	1.6 (±0.04)
Staminate spike length		12.9 (±0.41)	10.1 (±0.43)	10.8 (±0.43)
Staminate spike width		3.0 (±0.02)	2.6 (±0.03)	1.6 (±0.12)
Anther diameter		0.6 (±0.01)	0.5 (±0.01)	0.7 (±0.02)
Anther distance from tip		0.6 (±0.01)	0.5 (±0.02)	0.5 (±0.03)
Petal length		1.5 (±0.02)	1.4 (±0.02)	1.5 (±0.03)
Petal width		1.4 (±0.02)	1.2 (±0.03)	1.2 (±0.02)
Fruit length		5.4 (±0.04)	4.3 (±0.06)	4.5 (±0.05)
Fruit width		3.7 (±0.04)	3.0 (±0.04)	3.0 (±0.03)
Seed length		3.5 (±0.04)	2.4 (±0.04)	2.6 (±0.04)
Seed width		1.5 (±0.03)	1.1 (±0.02)	1.1 (±0.02)

Table 5. Canonical statistics: quadratic discriminant function analysis (DFA) of female (n= 8 morphological characters) and male plants (n= 10 morphological characters) using complete data or randomly selected records (n= 50 complete records/taxon) for *Arceuthobium campylopodum*, *A. laricis*, and *A. tsugense*. Can = Canonical, Cum % = cumulative %, Canonical r = canonical correlation, L Ratio = likelihood ratio,

Sex / Sample	**Can**	**Eigenvalue**	**%**	**Cum %**	**Canonical r**	**L Ratio**	**Approx *F***	***P*-value**
Female								
Complete	1	4.22	91.9	91.9	0.9	0.14	$F_{16, 1640} = 171.6$	< 0.0001
	2	0.37	8.1	100	0.52	0.73	$F_{7, 821} = 43.4$	< 0.0001
Random	1	4.24	83.3	83.3	0.9	0.1	$F_{16, 280} = 37.0$	< 0.0001
	2	0.85	16.7	100	0.68	0.54	$F_{7, 141} = 17.1$	< 0.0001
Male								
Complete	1	3.53	84.7	84.7	0.88	0.13	$F_{20, 2018} = 174.0$	< 0.0001
	2	0.64	15.3	100	0.62	0.61	$F_{9, 1010} = 71.7$	< 0.0001
Random	1	4.08	75.2	75.2	0.9	0.08	$F_{20, 276} = 33.9$	< 0.0001
	2	1.35	24.8	100	0.76	0.43	$F_{9, 139} = 20.8$	< 0.0001

Table 6. Quadratic discriminant function analyses (DFA) using complete data (complete) or randomly selected records (random; n= 50 complete records/taxon) for female and male plants of *Arceuthobium campylopodum*, *A. laricis*, and *A. tsugense*: standardized correlation coefficients. Anther diameter (AD); anther distance from tip (ADT); basal diameter (BA); fruit length (FL); fruit width (FW); petal length (PL); petal width (PW); plant height (PH); staminate spike length (SSL); staminate spike width (SSW); third internode length (TIL); and, third internode width (TIW).

Character	**Female (sample/canonical)**				**Male (sample/canonical)**			
	Complete		**Random**		**Complete**		**Random**	
	1	**2**	**1**	**2**	**1**	**2**	**1**	**2**
PH	0.26	0.41	0.21	0.31	0.08	0.50	-0.02	0.68
BD	-0.04	0.00	0.04	0.16	-0.19	0.20	-0.25	0.06
TIL	-0.21	0.67	-0.29	0.87	-0.35	0.16	-0.39	0.01
TIW	0.59	-0.75	0.37	-1.03	0.90	0.09	1.11	0.1
FL	0.38	0.37	0.51	0.43				
FW	0.12	-0.51	0.09	-0.44				
SL	0.39	0.25	0.39	0.10				
SW	0.28	0.04	0.38	0.01				
SSL					-0.13	0.20	-0.09	0.23
SSW					0.86	-0.42	0.72	-0.6
AD					-0.22	0.37	-0.27	0.32
ADT					-0.42	-0.01	-0.67	0.17
PL					-0.01	0.26	0.06	0.3
PW					0.18	0.00	0.17	-0.06

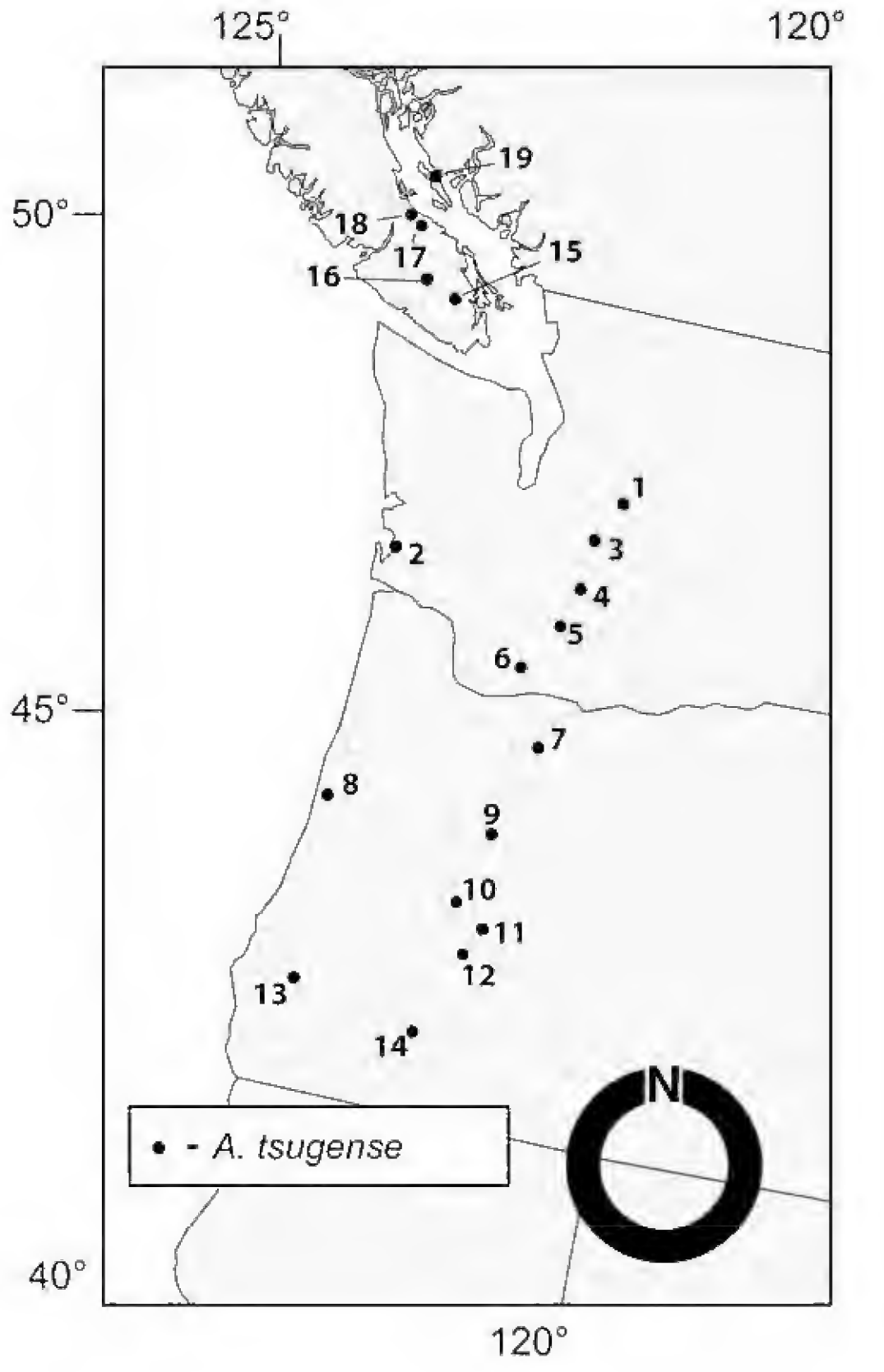

Figure 1. Approximate locations of collection sites for *Arceuthobium tsugense* in Washington, Oregon, and British Columbia. All collections on *Tsuga heterophylla*. **Washington**: **1** - Snoqualmie Pass, **2** - Westport, **3** - Huckleberry Creek, **4** - Cortright Creek, **5** - Clearwater Creek, **6** - Wind River Experimental Forest; **Oregon**: **7** - Wapinitia Pass, **8** - Desolation Saddle, **9** - Huckleberry Creek, **10** - Indigo Spring; **11** - Wall Creek, **12** - Calapooya Ridge, **13** - Union Creek, **14** - Iron Mountain; **British Columbia**: **15** - Holt Creek, **16** - Caycuse Summit, **17** - Spider Lake, **18** - Bowser, **19** -Texada Island.

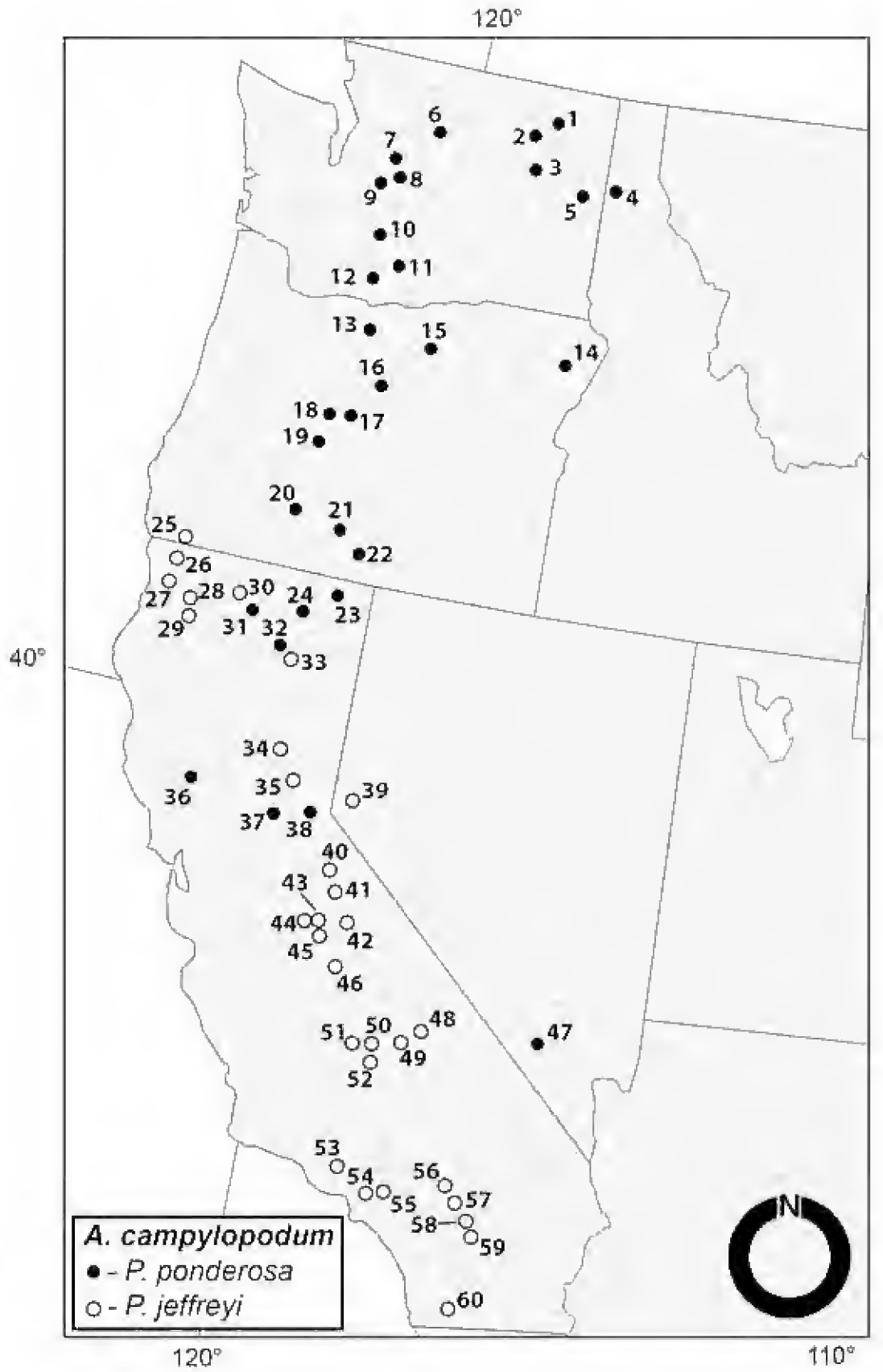

Figure 2. Approximate locations of collection sites for *Arceuthobium campylopdoum* in Washingtion, Idaho, Oregon, California, and Nevada. Closed circles present locations where plants were collected from *Pinus ponderosa*. Open circles represent locations where plants were collected from *P. jeffreyi*. Numbers correspond to the following locations: **Washington**: **1**- 4.5 km N of Gifford on St. Rte. 25, **2** - 20 km S of Fruitland on St. Rte. 25, **3** - 2 km NW of Nespelem on St. Rte. 155, **5** - 16 km S of Spokane on St. Rte. 195, **6** - 2.5 km W of St. Rte. 153 on Squaw Creek rd., **7** - Lake Wenatchee on Chiwawa River Loop rd., **8** - 2.6 km W of Squilchuck St. Park on road to Mission Ridge Ski Area, **9** - 0.8 km W of St. Rte. 97 on St. Rte 970, **10** - 17.6 km E of White Pass on St. Rte. 12, **11** - 2 km N of Satus Pass on St. Rte. 97, **12** - 3 km S of Trout Lake on St. Rte. 141; **Idaho**: **4** - 2.3 km N of Coeur d'Alene on Fernan Lake rd.; **Oregon**: **13** - 6.4 km W of Friend on forest rd. 27, **14** - 6.4 km S of Joseph on E shore of Wallowa Lk., **15** - 9.4 km on Sheep Cr. rd from forest rd. 51, Wallowa-Whitman Nat. For., **16** - 1.8 km E of Ochoco Summit on St. Rte. 26, **17** - 12.2 km W of St. Rte. 97 on St. Rte. 138, **18** - 15.2 km S of Sisters on forest rd. 16, **19** - 1 km from forest rd. 44 on forest rd. 4410, Pringle Falls Exp. For., **20** - Fort Klamath Cemetery on St. Rte. 62, **21** - 3 km W of Quartz Mtn. Pass on St. Rte. 140, **22** - Warner Mtn. Ski Hill on St. Rte. 26, **25** - 6 km S of Takilma on Greyback rd.; **California**: **23** - 3.4 km W of County rd. 48 on forest rd. 73, west shore of

Goose Lk., **24** - 16 km N of Adin on St. Rte. 299/139, **26** - 1 km S of forest rd. 17N26 on forest rd. 17N11, Klamath Nat. For., **27** - 6.2 km W of St. Rte. 96 on Dillon Mtn. rd., **28** - 9.6 km S of Callahan on St. Rte. 3, **29** - 10 km E of St. Rte 3 on forest rd. 17, Shasta-Trinity Nat. For., **30** - 2.4 km W of Stewart Hot Springs on forest rd. 17, **31** - 2 km N of St. Rte. 89 on Mt. Shasta Ski Park rd., **32** - 0.1 km S of St. Rte. 299 on St. Rte. 89, **33** - 2 km S of Old Station on St. Rte. 44, **34** - 2 km W of St. Rte. 44 on forest rd. 101, **35** - 14.4 km W of Susanville on St. Rte. 36, **36** - 19.5 km N of Upper Lake on Pillsbury Lk. rd., **37** - 7.7 km N of Pollock Pines on forest rd. 4, **38** - at entrance to Sugar Pine State Park, west shore of Lk. Tahoe, **40** - 1 km N of Markleeville on St. Rte. 89, **41** - Silver Creek Campground on St. Rte. 4, **42** - Column of the Giants on St. Rte. 108, **43** - Pinecrest Transfer Station 0.5 km W of Pinecrest on St. Rte. 108, **44** - 1 km W of Long Barn on St. Rte. 108, **45** - 8.5 km E of Crane Flat on St. Rte. 120, **46** - 2 km W of Big Creek on rd. to Shaver Lk., **48** - 8.5 km W of Sherman Pass on forest rd. 22S05, **49** - 2.2 km S of Troy Mdws. Campground, Sequoia Nat. For., **50** - 5.8 km N of rd. to Johnsonville on Western Divide Highway, **51** - Pine Flat, Sequoia Nat. For., **52** - Tiger Flat, Sequoia Nat. For., **53** - 6.2 km S of St. Rte. 33 on rd. to Mt. Reyes, **54** - 1.4 km W of Cloud Burst on St. Rte. 2, **55** - 1 km W of Big Pines on St. Rte. 2, **56** - 2.4 km N of Fawnskin on forest rd. 2N71, **57** - 1.9 km from St. Rte. 38 on rd. to Jenks Lk., **58** - near Ranger Station in Idylwild, **59** - 1.1 km S of the S Fork San Jacinto River Bridge on St. Rte. 74, **60** - 0.5 km S of Horse Heaven Campground on Sunrise Highway; **Nevada**: **39** - Bowers Mansion St. Park, **47** - 4.1 km W of Ranger Station at Old Ski Tow Historic Site, Kyle Canyon.

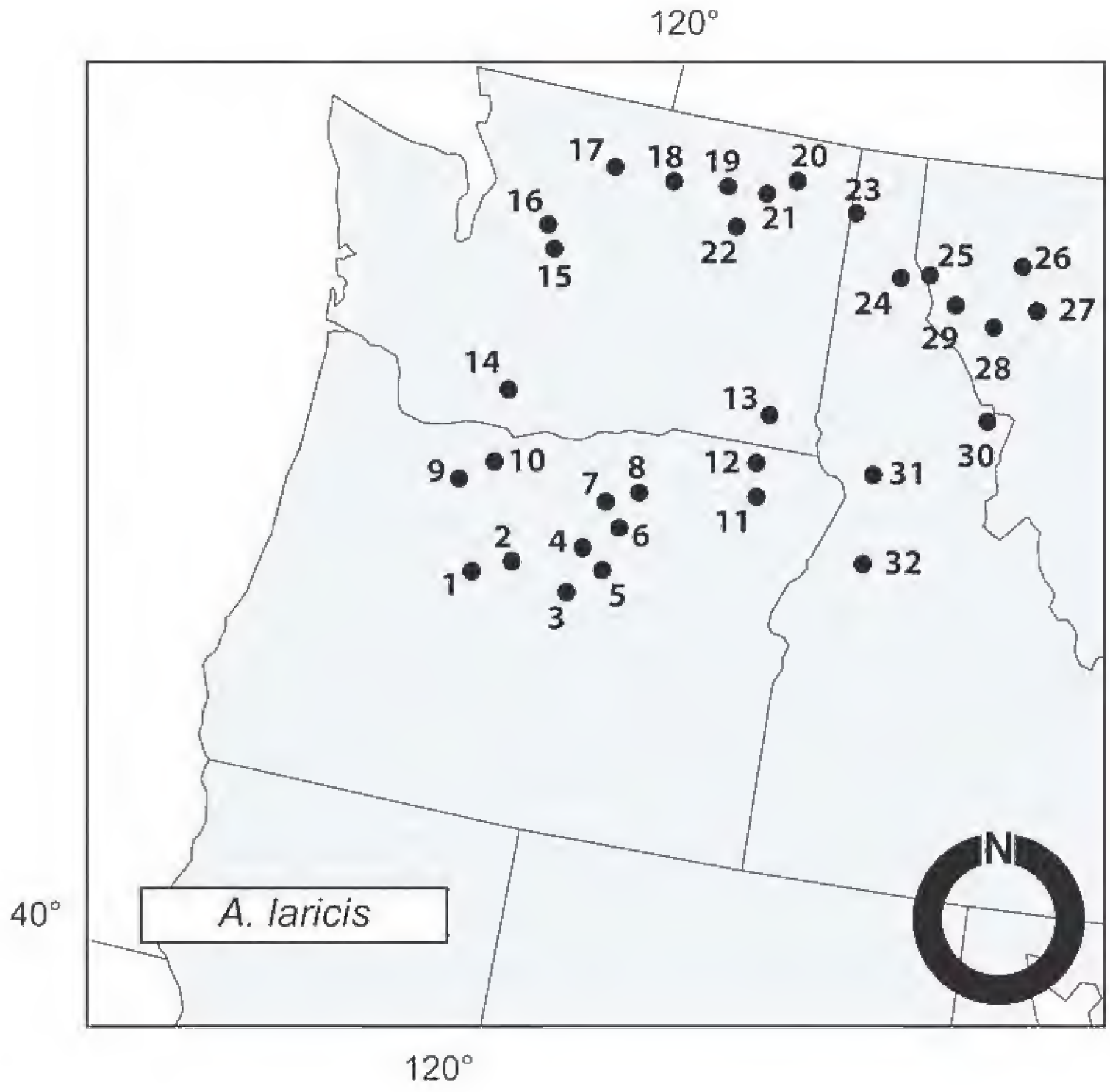

Figure 3. Approximate locations of collection sites for *A. laricis* in Washington, Oregon, Idaho, and Montana. All collections on *Larix occidentalis*. **Oregon**: **1** - Camp Sherman, **2** - Ochoco Summit, **3** - S of John Day, **4** - Blue Mountain Pass, **5** - Dixie Pass, **6** - N of Sumpter, **7** - NW of Granite, **8** - Sheep Creek, **9** - Skyline Road, **10** - Marion Point, **11** - S of Lostine, **12** - N of Enterprise; **Washington**: **13** - Fields Spring State Park, **14** - Lost Creek, **15** - Mission Ridge, **16** - S of Blewett Pass, **17** - Loup Loup Summit, **18** - Disautle Pass, **19** - Pass Creek, **20** - Tiger Meadows, **21** - S of Fruitland, **22** - E of Colville; **Idaho**: **23** - S of Coolin, **24** - Thompson Pass, **30** - W of Lolo Pass, **31** - Snow Haven Ski Area, **32** - Ponderosa State Park; **Montana**: **25** - E of Thompson Pass, **26** - S of Big Fork, **27** - E of Glacier Lake, **28** - N Valley Creek, Flathead Indian Reservation, **29** - E of Cooper Pass.

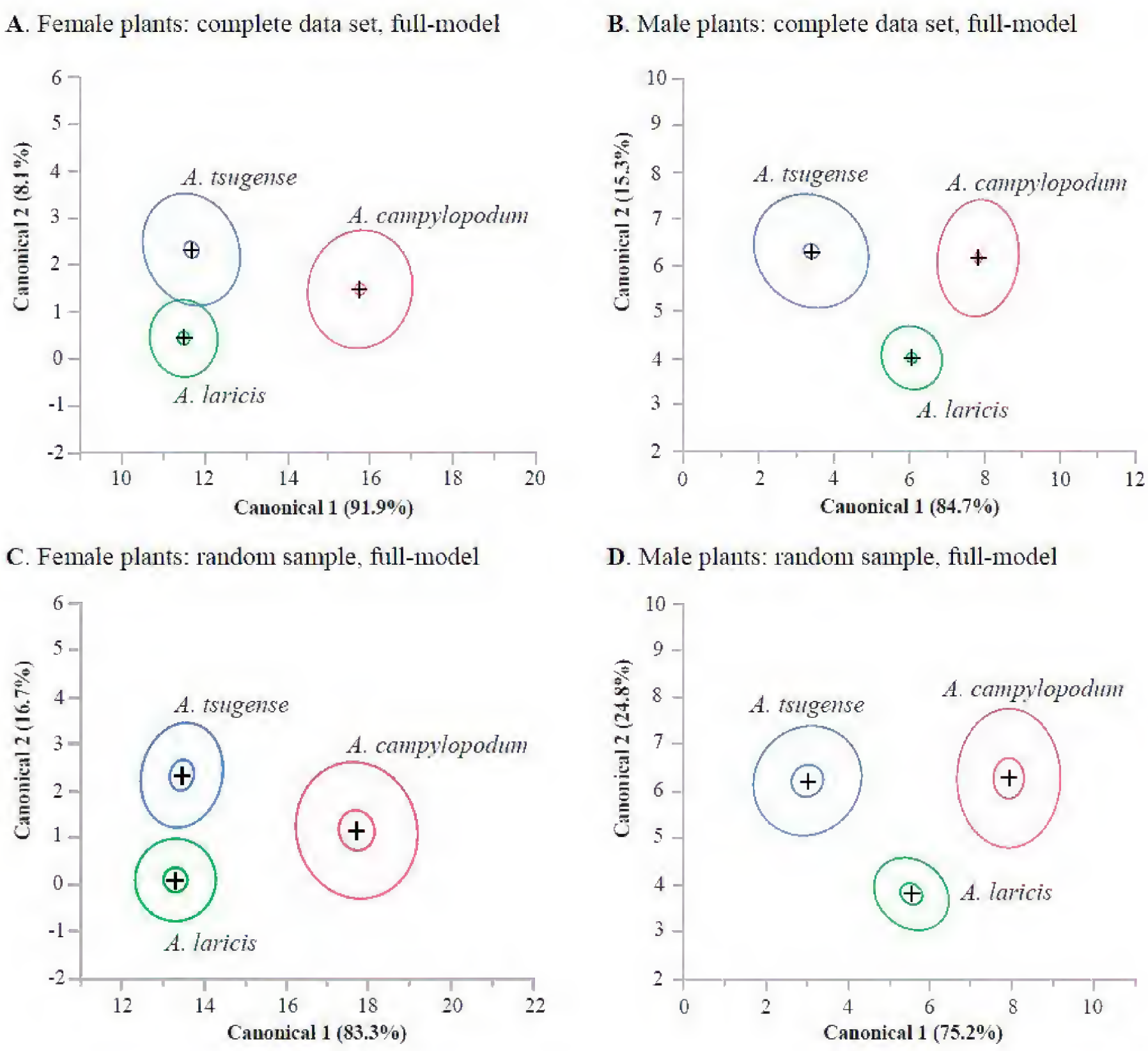

Figure 4. Canonical plots for discriminant function analyses (DFA) of *Arceuthobium campylopodum*, *A. laricis*, and *A. tsugense* based on morphological characteristics of female (A, C) and male plants (B, D) shown in Table 6. Multivariate means (crosshairs) were computed using complete data for each species by sex (A, B), whereas, to further validate the DFA, means were also calculated using a random subset (50 complete records/species) of female (C) and male plants (D), respectively. For each species (A-D), the inner ellipse correspond to a 95% confidence limit for the mean, and the outer ellipse represent a normal 50% contour illustrating the approximate area within which 50% of plants for each species reside.

Nomenclatural corrections in Convolvulaceae diversae.

G.W. Staples
HRA, Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, U.K.
email: geozsg@gmail.com

Maria Teresa Buril
Área de Botânica - Departamento de Biologia, Universidade Federal Rural de Pernambuco
Rua Dom Manoel de Medeiros s/n, Recife – PE, CEP: 52171-030, Brazil

Ana Rita Simões
Instituto de Botânica de São Paulo, Herbário, Av. Miguel Stéfano 3687,
Água Funda, São Paulo, SP, CEP 04301-902, Brazil

and

Rafaël Govaerts
Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, U.K.

ABSTRACT

New combinations and new reductions to synonymy are reported here for 11 Convolvulaceae so that nomenclaturally satisfactory names are available for use in the on-line World Checklist of Convolvulaceae. The new names and new combinations replace names in current use that were found to be illegitimate or had earlier epithets available that must be taken up to comply with the Code. Published on-line www.phytologia.org *Phytologia 97(3): 219-223 (July 1, 2015).* ISSN 030319430.

KEY WORDS: *Astripomoea, Ipomoea, Jacquemontia, Merremia,* new combinations, plant nomenclature

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In working through more than 8,500 published scientific names for a world checklist of Convolvulaceae, a number of nomenclatural problems were encountered. Some of these are resolved here to make corrected names and new combinations available for use in the recently completed family checklist that is available now on the World Checklist of Selected Plant Families site (http://apps.kew.org/wcsp/). In other cases, species have been resurrected from synonymy or forgotten names have been resurrected that displace names in current use, and the relevant synonymies are succinctly summarized.

Astripomoea A.Meeuse

Astripomoea malvacea var. **parviflora** (Rendle) Staples, *comb. nov.*

≡ *Astrochlaena stuhlmanii* var. *parviflora* Rendle, Fl. Trop. Africa Convolv. 4(2): 122. 1905. Type: [country unknown] "Nile Land" Taruma, near Tulpanga, *Kässner 437* (syntype BM); Tanzania, plateau north of Lake Nyasa, *Thomson s.n.* (syntype K).

= *Astripomoea malvacea* var. *volkensii* (Dammer) Verdc. (1958), *nom. superfl., syn. nov.*

Verdcourt (1958: 193) should have taken up the earlier epithet available at varietal rank instead of making a new combination based on *Astrochlaena volkensii* Dammer (1895) and reducing Rendle's variety under it.

Ipomoea L.

Ipomoea capitellata Choisy, Mém. Soc. Phys. Genève 6: 457 [Convolv. Or. 75] 1834.
Type: Nepal, Mungger, 5 Oct. 1811, *N. Wallich sub Wallich Cat. 2250* (holotype G-DC!; isotype K-W!).

= *Ipomoea bracteata* Wight, Icon. Pl. Ind. Orient. 4(2): 14, t. 1374. 1848, non Cav. (1799). Type: India, [Kerala] Quilon, anno 1837, *R. Wight '2383'* (isotypes K[x2]!).

≡ *Ipomoea deccana* D.F.Austin, Rev. Handbk. Fl. Ceylon 1: 324. 1980, *syn. nov.* Type: based on same type as Wight's name.

Ipomoea capitellata disappeared in synonymy almost a century ago under *Ipomoea pes-tigridis* L., based on Hallier's taxonomic concept, which combined entire-leaved plants and deeply palmately lobe-leaved plants under a single species. In the course of nomenclatural review for the World Checklist of Convolvulaceae it became clear that two distinct species are involved, and Choisy's name for it is much older than Austin's later replacement name. Noltie (2005) found no Wight specimens in E that could be type material for *I. bracteata* Wight; two sheets of original material were discovered in Kew. The diagnostic features are the entire, cordiform leaves, capitate inflorescences with ovate bracts, distinctly cordate basally.

Ipomoea descolei O'Donell, Lilloa 23: 440. 1950.

= *Argyreia choisyana* Regel & Körn., Index Semin. (LE) 1858: 40. 1859, *non* Wight ex C.B.Clarke (1883). Type. Russia. cultivated in the St. Petersburg Botanic Garden (holotype, LE!) , *syn. nov.*

The Regel and Körnicke name has long been problematic because it predates the later *A. choisyana* (Wight) Wight ex C.B. Clarke (1883), which has often been used in floras and Indian botanical literature, despite being illegitimate. Discovery of original material for *A. choisyana* Regel & Körn. in the LE herbarium in 2013 finally solved the puzzle: it is a South American species, not an Asian one, and because the epithet is blocked in *Ipomoea*, it becomes a synonym of the later name, *I. descolei*.

Ipomoea pes-tigridis L. var. **africana** Hallier f., Bull. Herb. Boissier 6: 539. 1898.

= *Ipomoea pes-tigridis L.* var. *strigosa* (Hallier f.) Baker & Rendle in Dyer, Fl. Trop. Africa 4(2): 159. 1905, *syn. nov.*

Lejoly and Lisowski (1992: 47), in their account of central African *Ipomoea*, should have taken up the earlier name at varietal rank, which they listed in synonymy, rather than var. *strigosa*, which post-dates it.

Ipomoea peteri (Kuntze) Staples & Govaerts, *comb. nov.*

≡ *Mouroucoa peteri* Kuntze, Revis. Gen. Pl. 3(2): 218. 1898 ("*Murucoa*"), *nom. nov.*

≡ *Ipomoea sericophylla* Peter in Engl. & Prantl, Nat. Pflanzenfam. 4(3a): 31. 1891 non Meisn. (1869). Type: Guatemala, Zacatal, *Bernoulli & Cario 1892* (lectotype GOET005709).

= *Ipomoea tuxtlensis* House, Ann. New York Acad. Sci. 18: 256. 1908. *syn. nov.*

Staples et al. (2012: 676) resolved the disposition of the Peter Convolvulaceae names, including *I. sericophylla* Peter, but at the time it was not apparent that *Mouroucoa peteri* Kuntze is an older name for the species known as *I. tuxtlensis* House and a new combination in *Ipomoea* is required.

Ipomoea ternifolia var. **villosa** (Choisy) Staples & Govaerts, *comb. nov.*

≡ *Ipomoea muricata* Cav. var. *villosa* Choisy in De Candolle, Prodr. 9: 353. 1845. Type: Mexico. Cuernavaca, 20 Oct. 1827, *Berlandier 974* (lectotype, designated here, G [G00135571]!).

= *I. ternifolia* var. *leptotoma* (Torr.) J.A. McDonald, Harvard Pap. Bot. 6: 120. 1995, *nom. superfl.*
≡ *Ipomoea leptotoma* Torr., Bot. Mex. Bound. 150. 1859, *syn. nov.*

= *Ipomoea ternifolia* var. *wootonii* Kelso, Rhodora 39: 151. 1937, *syn. nov.*

McDonald (1995: 120) should have taken up the varietal name *villosa* instead of making a new combination based on a name at specific rank.

Jacquemontia Choisy

Jacquemontia breviacuminata (Mart. ex Choisy) Buril, *comb. nov.*

≡ *Convolvulus breviacuminatus* Mart. ex Choisy in De Candolle, Prodr. 9: 409. 1845. Type: Brazil. Piauhi: in campis prope Campo-Grande et Castello praedia, *Martius Obs. no. 2459* (holotype M).

= *Jacquemontia racemosa* Meisn. in Martius, Fl. Bras. 7: 308. 1869, *nom. illeg.*

Meisner coined a new name in *Jacquemontia* while citing an older name, *C. breviacuminatus*, in synonymy with it, thereby making his new name illegitimate. The error is corrected here by taking up the earliest available epithet in *Jacquemontia* now.

Jacquemontia cephalantha Hallier f., Jahrb. Hamburg. Wiss. Anst. 16(Beih. 3): 30. t.p. 1898, publ. 1899.

≡ *Ipomoea cephalantha* Dammer, Bot. Jahrb. Syst. 23(Beibl. 57): 39. 1897, non Baker (1894). Type: Brazil: civitate São Paulo ad Serra da Bocaina in campo, *Glaziou 19670* (syntype B†; isosyntypes K, R); civitate Minas ad Biribing prope Diamantina, *Schwacke 8206* (syntype B†).

≡ *Jacquemontia hallieriana* Ooststr., Recueil Trav. Bot. Néerl. 33:216. 1936. *nom. superfl.*

Ooststroom rejected the name *J. cephalantha* on the grounds that its basionym is illegitimate and coined a replacement name. The modern ICN however, allows the epithet *cephalantha* to be used in *Jacquemontia* and interpreted as a new name, credited to Hallier, and dating from his publication. Taking this route means that the avowed replacement name is superfluous.

Jacquemontia gabrielii (Choisy) Buril, *comb. nov.*

≡ *Ipomoea gabrielii* Choisy in De Candolle, Prodr. 9: 378. 1845. Type: [French Guiana] Cayenne, *Gabriel s.n.* (holotype G).

= *Jacquemontia ciliata* Sandwith, Bull. Misc. Inform. Kew 1930: 156. 1930, *syn. nov.*

The type specimen of *I. gabrielii*, once located in the Geneva herbarium, proved to be a *Jacquemontia* and conspecific with the species long known as *J. ciliata,* a much later name.

Merremia Dennst. ex Endl.

Merremia grandidentata (C.H. Thomps.) Staples & Simões, *comb. nov.*

≡ *Ipomoea grandidentata* C.H. Thomps., Trans. Acad. Sci. St. Louis 20: 18. 1911. Type: U.S.A. Missouri: St. Louis, cultivated in Missouri Botanical Garden, Oct.-Nov. 1980, *C.H. Thompson s.n.* (syntype MO!).

This species, under a name long overlooked in *Ipomoea*, clearly belongs to the genus *Merremia s.l.*, which will soon be broken up into segregate genera. By making a new combination for it now we draw attention to the species, place it where it belongs, and point out the need for collectors in Mexico to locate the wild populations and gather material for further study. The protologue states that the seeds cultivated in the Missouri Botanical Garden glasshouse came from a plant that originated in "Torreon, Mexico" (Coahuila state). It is unfortunate that Thompson (1911: 19) did not accept the opinion of Hans Hallier, mentioned in the protologue, that this species is a genuine *Merremia,* for that is where it belongs.

Merremia martini (H.Lévl.) Staples & Simões, *comb. nov.*

≡ *Ipomoea martini* H.Lévl., Repert. Spec. Nov. Regni Veg. 9:453. 1911. Type: China. [Guizhou] Kouy-Tcheou, env. de Gan-pin, 9 Sept. 1897, *L. Martin sub E. Bodinier 1806* (holotype E!).

≡ *Ipomoea wilsonii* Gagnep., Not. Syst. 3: 150. 1915, *nom. illeg., syn. nov.*

= *Merremia hungaiensis* (Lingelsh. & Borza) R.C.Fang, in Fl. Reipubl. Popul. Sin. 64(1): 76. 1979.
≡ *Ipomoea hungaiensis* Lingelsh. & Borza, Repert. Spec. Nov. Regni Veg. 13: 389. 1914, *syn. nov.*

It was pointed out many years ago by Launert (1979: 145) that *I. martini* is conspecific with *I. hungaiensis*, but the new combination in *Merremia* was not made then and the note was forgotten until the literature search for the WCSP compilation unearthed it. *Bodinier 1806* is also a syntype for *I. wilsonii*, which makes this name superfluous and illegitimate.

ACKNOWLEDGEMENTS

The American Philosophical Society awarded funds in 2007 to initiate the World Checklist and Bibliography of Convolvulaceae. Olga Cherneva (LE) kindly searched for the type specimen of *Argyreia choisyana* and sent digital images of it for analysis. We thank J. Andrew McDonald (PAUH) and John Wiersema (BARC) for their prompt and constructive reviews, which pointed out several areas for improving the manuscript.

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Nomenclatural Note:

Validation of the names *Senegalia x turneri*, *S. x zamudii*, and *Vachellia x ziggyi*

David S. Seigler
Department of Plant Biology, University of Illinois, Urbana, Illinois 61801
seigler@life.illinois.edu

John E. Ebinger
Emeritus Professor of Botany
Eastern Illinois University, Charleston, Illinois 61920

and

Catherine Glass
Pacific Northwest College of Art, Portland, Oregon 97209

KEY WORDS: *Senegalia x turneri* Seigler & Ebinger, nothospecies nov. (*Senegalia berlandieri* x *S. wrightii*), *Senegalia x zamudii* Seigler, Ebinger & Glass, nothospecies nov. (*Senegalia berlandieri x S. reniformis*), *Vachellia x ziggyi* Seigler & Ebinger, nothospecies nov. (*Vachellia collinsii* and *V. pennatula*),name validation. Published on-line www.phytologia.org *Phytologia 97(3): 219. (July 1, 2015).* ISSN 030319430.

Recently we (Seigler, Ebinger & Glass 2012, 2013; Seigler & Ebinger 2013) published "*Senegalia x turneri* Seigler, Ebinger & Glass", "*Senegalia x zamudii* Seigler & Ebinger" and "*Vachellia x ziggyi* Seigler, Ebinger & Glass" as new hybrid taxa. The protologue of each includes a description in English, listing of the names of the hybrid parents, and holotype citation. Unfortunately, as an act of oversight, we cited the rank of the hybrid taxon as "nothomorph" (= variety; vide Melbourne Code Art. H12.2; McNeill & al. 2012).

Although our intention was to flag the binomial as "nothospecies", we inadvertently used the misplaced rank term "nothomorph", and thus the names "*Senegalia x turneri* Seigler, Ebinger & Glass", "*Senegalia x zamudii* Seigler, Ebinger, & Glass:, and *Vachellia x ziggyi* Seigler & Ebinger were not validly published (Arts. 5, 37.6). We herewith correct the mistake and validate these names.

Senegalia x turneri Seigler, Ebinger, and Glass, **nothospecies nov.** (*Senegalia berlandieri* x *S. wrightii*). TYPE: UNITED STATES. TEXAS: Uvalde Co.: Harris Ranch near Cline, 20 miles W of Uvalde on route 90, 18 Aug 2003, ***D.S.Seigler & J.E.Ebinger 15815*** (ILL).

Senegalia x zamudii Seigler, Ebinger & Glass, **nothospecies nov.** (*Senegalia berlandieri x S. reniformis*). TYPE: MEXICO. Querétaro. Cañón del Río Extorax entre El Plátano y El Timbre, 900 m, 12 Dec. 1999, ***S. Zamudio, E. Esparza & E. Zamudio 11241*** (holotype, MEXU, photo of MEXU at ILL).

Vachellia x ziggyi Seigler & Ebinger, **nothospecies nov.** (*Vachellia collinsii* x *V. pennatula*). TYPE: MEXICO. Oaxaca. A 5 km al NE de San Pedro Tepanatepec, Distr. Juchitán, 200 m, 16 Dec 1978, ***M. Sousa, L. Rico & P. Basurto 10157*** (holotype: MO).

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Taxonomy of Chamaesaracha (Solanaceae)

Billie L. Turner
Plant Resources Center,
University of Texas, Austin, TX 78713 U.S.A.
billie@uts.cc.utexas.edu

ABSTRACT

Chamaesaracha is a commonly encountered genus of perennial herbs largely confined to Texas and north-central Mexico. Averett (1973), as part of a Doctoral Thesis, monographed the genus; in this he recognized the generitype, *C. coronopus*, and six other species: *C. coniodes, C. sordida, C. edwardsiana, C. pallida, C. villosa* and *C. crenata*, but excluded *C. potosina*. Hunziker (1980) accepted the latter and proposed two additional species: *C. cernua* and *C. rzedowskiana*, and added two more in 1995, *C. viscosa* and *C. spruceana*; Averett (2005) described *C. darcyii* and yet another *C. geohintonii* (Averett 2010). Henrickson (2009) added *C. arida* and *C. texensis*. My reevaluation of the genus has resulted in the recognition of 10 species, but with a revised structuring and nomenclature, as follows: *C. arida, C. coniodes* (including *C. texensis*), *C. coronopus, C. crenata, C. edwardsiana, C. felgeri* B.L. Turner, **sp. nov.,** *C. geohintonii, C. pallida, C. rzedowskiana,* and *C. sordida. Chamaesaracha cernua, C. potosina, C. spruceana* and *C. viscosa* belong with other genera and are excluded. Photographs of the types of the newly described taxa are provided. A key to the taxa and distribution maps for all of the species is presented, along with observations relative to the dispositions made. Published on-line www.phytologia.org *Phytologia 97(3): 226-245 (July 1, 2015).* ISSN 030319430.

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Averett (1973) published a taxonomic account of the genus **Chamaesaracha**, this part of his doctoral studies. In this, he recognized seven species, as indicated in the above abstract. Hunziker (1980, 1995) proposed five additional species: **C. rzedowskiana**, **C. cernua**, **C. potosina**, **C. viscosa** and **C. spruceana**; Averett (2005) and Averett (2010), added an additional two species, **C. darcyii** and **C. geohintonii**.

The present author first became interested in the complex while working with Averett to identify Texas material for his Atlas of Texas Plants (Turner 2003). He and yet other workers had found the species of **Chamaesaracha** highly variable and keys for their recognition difficult to use. Independently, Averett had started to reexamine some of the taxa, having had doubts about several of the species added to the genus by Hunziker. Subsequent field studies and herbarium work over a several year period, by both Averett and myself, has led to the recognition of additional species of **Chamaesaracha**, results of this activity presented below.

CHAMAESARACHA (A. Gray) Benth. & Hook.

Overall, my concept of the genus is about the same as that of Averett (1973), with the exception that I include **C. rzedowskiana** in the genus. With the addition of this very distinctive element, and the recognition of **C. felgeri** (described below) the only expansion to the generic description of **Chamaesaracha** is that of habit: the genus now includes an annual or weak perennial species with prostrate stems, the latter rooting at the nodes. Because of this I have not felt the necessity to describe again **Chamaesaracha**: Averett's presentation seems adequate. Following Averett, I have excluded from the genus, *C. potosina*, *C. cernua*, *C. viscosa* and *C. spruceana*.

I believe the information presented herein will help clarify the taxonomy of **Chamaesaracha** but it remains a taxonomically difficult group of several similar, highly variable species. For example, pubescence, as in **Physalis** (Seithe and Sullivan, 1990), is an important taxonomic character within **Chamaesaracha** but may be under relatively simple genetic control. This likelihood is amply attested to by the work of Oppenheimer et al. (1998) who found that a single gene could account for the production of dendritic hairs in the genus **Arabidopsis**. Such simple genetic mechanisms may account for some of the variability observed among the species of **Chamaesaracha**. Indeed, leaf and stem vestiture in the latter is fairly, but almost never completely, consistent within a taxon and must be used in conjunction with other characters, especially leaf shape, when attempting identifications. With the exception of **C. rzedowskiana**, no species exhibits a unique character but, rather, each possesses a unique combination of characters. This may make my key to species difficult to use, so one should utilize both geography and the more complete descriptions of each of the species so as to assure identification.

Key to species

1. Plants strictly prostrate, the stems markedly zig-zag and rooting at nodes; easternmost San Luis Potosi and Queretaro .....**C. rzedowskiana**
1. Plants variously procumbent to erect, not rooting at the nodes...(**2**)

2. Corollas, when flared, 2-3 cm across; mid-stem leaves mostly 7-12 cm long; sepals pubescent with elongate dendritic hairs; deep sandy soils of Yuma Co., Arizona.....**C. felgeri**
2. Corollas, when flared, 1.0-2.0 across; mid-stem leaves mostly 2-6 cm long; sepal pubescence otherwise...(**3**)

3. Pedicels 5-15 mm long; stem-hairs mostly 1-2 mm high, beneath a dense array of glandular hairs 0.3-0.4 mm high.....**C. sordida**
3. Pedicels mostly longer, the stem-hairs various; widespread…(4)

4. Mid-stem leaves mostly 1.5-3.5 cm wide, their vestiture mostly composed of elongate trichomes with an understory of short glandular hairs, a few dendritic hairs interspersed among these.....**C. crenata**
4. Mid-stem leaves mostly 0.3-1.5(2.5) cm wide, variously pubescent or glabrate, but not as described in the above…(**5**)

5. Mid-stems glabrous to variously pubescent with dendritic hairs, the latter often intermixed with a smattering of smaller glandular hairs...(**7**)
5. Mid-stems markedly viscid with glandular hairs, the latter often over-topped by elongate simple trichomes, or these intermixed with a smattering of short dendritic or branched hairs…(**6**)

6. Plants forming nearly prostrate mats ca 2 ft across; leaf-blades ovate, markedly lobate, rarely not; roots fleshy, 6-10 mm thick; USA, e Chi, Coa, e Dur…….....**C. crenata**
6. Plants forming erect clumps; leaf blades mostly unlobed, or nearly so; roots slender, 2-5 mm thick; widespread…….....……**C. sordida**

7. Leaves glabrous or nearly so; Edwards Plateau of central Texas…….....…… **C. edwardsiana**
7. Leaves variously pubescent (except for C. pallida); widespread…(**8**)

8. Mid-stem leaves mostly entire (rarely not); Trans-Pecos, Texas, southern New Mexico and north-central Mexico.....**C. pallida**
8. Mid-stem leaves markedly lobate or closely sinuate (rarely not)…(**9**)

9. Mid-stem leaves various, but not usually as described below; pubescence varied, but not white-tufted or cottony-like…(**11**)
9. Mid-stem leaves markedly linear-lobate, usually dissected (rarely not)…(**10**)

10. Pubescence composed of peculiar, short, white-tufted dendritic hairs.....**C. arida**
10. Pubescence composed of longer, stellate and/or dendritic trichomes, occasionally glabrate; s Texas and ne Mexico .....**C. coronopus**

11. Leaves flabellate with lobed margins, rarely entire; pubescence an array of dendritic hairs 1-2 mm high; Mexico and western Texas .....**C. crenata**
11. Leaves and/or pubescence not as described in the above…(**12**)

12. Leaves moderately to heavily stipitate-glandular (occasionally with stellate hairs in a few populations, esp. in southern Texas); widespread.....**C. coniodes**
12. Leaves pubescent with dense, eglandular, dendritic hairs; USA, north-central Tex and sw Okla.....**C. darcyi**

CHAMAESARACHA ARIDA Henrickson , Phytologia 91: 186. 2009.
Maps 1a, 1b

TYPE: **USA. NEW MEXICO: Santa Fe Co.**, 19 mi S of Santa Fe, *Averett & Tomb 339* (Holotype: TEX)

Erect rhizomatous perennial 6-20 cm high. Stems sparsely to moderately pubescent with distinctive short-branched cottony-like hairs. Leaves mostly linear-lanceolate, 3-8(10) cm long, 3-15 mm wide, mostly 4-6 times as long as wide, their margins remarkably lobate, rarely not. Flowers single, axillary, the peduncles (at anthesis) mostly spreading, 1-4 cm long. Calyces (flowering) 4-5 mm long, the lobes 2-3 mm long, pubescent like the stems. Corollas pale yellow or "yellow-green," 15-20 mm across the expanded corolla. Fruits a berry, orbicular, 6-8 mm across, containing 30-50 seeds. Seeds, ca 2 mm long, 1.5 mm wide. Chromosome number, n = 24 pairs.

Averett (1973) included this taxon under his broad concept of **C. coronopus**, as had earlier workers. Henrickson (by annotation) long treated the taxon as but a variety of **C. coronopus**, but, presumably, after noting the treatment of his proposed variety as specifically distinct (through annotations by Turner at LL-TEX), he also accepted its present status. Typical **C. coronopus** occurs largely to the east of **C. arida**. The two taxa are readily recognized by both vestiture and to some extent by leaf shape. Following Henrickson, and the present author, most of the references to **C. coronopus** in floristic literature for West Texas, New Mexico, Colorado, Arizona, and California would now be **Chamaesaracha arida**. Averett (2009), however, demurs noting that:

> Most, but not all, of the western populations of *C. coronopus*, as treated by Averett (1973), do have the short, branched hairs Henrickson described, but other consistent differences from these and populations of *C. coronopus* in south Texas are not found. Moreover, equal or greater differences are seen among several populations in the western United States and northern Mexico. I see little justification for the recognition of *C. arida* as a separate species but, with further analyses, it plausibly might be recognized at the varietal level.

Herein, I accept the specific status bestowed by Henrickson, this after treating the taxon as such myself, while Henrickson preferred varietal status, to judge from his early annotations of the complex.

CHAMAESARACHA CONIODES (Moricand ex Dunal) Britt., Mem. Torrey Bot. Club 5: 287. 1895. **Maps 2a, 2b**
Solanum coniodes Moricand ex Dunal in DC., Prod. 13: 64. 1852.

TYPE: **USA. TEXAS**: "inter Laredo et Bejar [San Antonio]," Feb-Mar 1828, *Berlandier exs. 1494.* (lectotype G-DC, selected by Averett, 1973; isolectotype GH!). Dunal, in his protologue, cited two collections by Berlandier, *1463* and 1494. The former was apparently collected along San Miguel Creek in what is now eastern Frio or southwestern Atascosa counties, the latter in March of 1828 from some verdant river site between San Miguel and San Antonio, to judge from label data at GH. Both collections are on the type sheet at GH.

[Averett (2010b) correctly notes that both Henrickson and Turner (by annotation 1997) misidentified the above type as **C. coronopus**, an error on my part due to ignorance, at the time, of the morphological parameters and distribution of the latter. I now agree with Averett's assessment.]

Solanum linsecumii Buckl., Proc. Acad. Nat. Sci. Philadelphia 1862: 6. 1863.
TYPE: **USA. TEXAS. Llano Co**., w/o specific locality, "June," w/o year, *Buckley s.n.* (holotype PH!). The type has unusually broad leaves, but possesses mostly slender branched hairs on its stem and peduncles, much as a somewhat glabrous form of **C. coronopus**.

Chamaesaracha texensis Henrickson, Phytologia 91: 187. 2009.
TYPE: **USA. TEXAS. Kinney Co,** "open rocky soil near the Nueces River, Hy. 334," 17 Apr, 1957, *D.S. Correll 15965* [with Rollins & Chambers] (Holotype: LL-TEX).

In his original description of *C. texensis*, Henrickson stated "From *Chamaesaracha sordida* distinguished by the young leaves being irregularly toothed, lacerate to pinnatifid, with entire to toothed lobes (not entire to bluntly or shallowly few toothed and tetraploid (n=24) not diploid n=12)."

Averett (2009) also considered Henrikson's *C. texensis* as belonging to the **C. coniodes** complex, but noted that Gray (1876) treated **C. coniodes** as synonymous with **C. sordida**, a treatment that was followed by Correll and Johnston (1970), this emended by Johnston (1990). Britton (1895), however, included the latter name under **C. coniodes**, this treatment followed by Rydberg (1896) [the latter misspelling it as "C. conioides."] The two species, however, are quite distinct and were recognized as such by Averett (1970, 1973, 2010b), whose views are followed here. Relevant to the following discussion, it should be noted that neither taxon has ever been confused with **C. coronopus**, except for the above errors in typification of **C. conioides** by both Henrickson and Turner.

Chamaesaracha coniodes is the most variable species of the genus, exhibiting essentially all forms of pubescence and a variety of leaf shapes. It also varies from robust plants to those relatively small in stature and occurs in a variety of habitats. Unfortunately, the type has a dense covering of dendritic hairs (as also noted by Averett, 2010b), atypical, but not unknown in the species, especially as it occurs to the north and west of the Edwards Plateau of Texas; otherwise it is comparable to **C. coniodes**. Henrickson (2009) recognized the more southern and southwestern elements as a new species, *C. texensis*. However, the leaves of the type of **C. coronopus** are 4-6 times as long as wide (as they typically are in **C. arida**) and essentially glabrous as are most of the populations in south Texas; those of **C. coniodes** are densely pubescent; hairs on the type of the latter are dense and are described as stellate but might best be described as short-dendritic or branching; further, the leaves of the type are ca 2.5 times as long as wide, and lack the deep lobed margins found in **C. coronopus**. In short, the types of **C. coronopus** and **C. coniodes** differ in leaf shape and pubecence, but share yet other features.

Finally, it should be noted that variation in **C. conoides** is likely compounded by hybridization with **C. sordida**, the two taxa sympatric over much of their distributions; likely contaminants in Texas include: Coke Co., *Hansen 5732*; Garza Co., Averett 357; San Saba Co., *Henderson 8*; Tom Green Co., *Watson 192*; Webb Co., *Crockett 6431*[all LL-TEX].

CHAMAESARACHA CORONOPUS (Dunal) A. Gray, Bot. Calif. 1: 54. 1876. **Map 3**
Solanum coronopus Dunal in DC., Prod. 13: 54. 1852.
Withania coronopus (Dunal) Torr., Bot. Mex. Bound. Surv. 155. 1859.
Saracha coronopus (Dunal) A. Gray, Proc. Amer. Acad. Arts 10: 62. 1874.

TYPE: **USA. TEXAS: Bexar Co**., "inter Laredo et Bejar [San Antonio]," Mar (?) 1828, *Berlandier exs. 1513* (holotype G-DC!). Averett (1973) noted the holotype as having been collected in the vicinity of Laredo, Texas, by Berlandier, citing his collection number *1494*; he subsequently corrected this (Averett, 1974) and by the inclusion of an informal insert in his distributed reprints. Only a single collection was cited by Dunal in his prologue, this being *Berlandier exs. 1513*. Averett did examine an isotype at K, noting that the locality on that sheet gave the locality as "Rio Medina," which on Berlandier's route would be in present-day Bexar Co., Texas.

My concept of **C. coronopus** includes those specimens of **Chamaesaracha** having a predominantly small habit, narrow leaves that are typically 4-6 times as long as wide, more deeply and regularly lobed than those of **C. arida**, and a pubescence of mostly slender branched hairs. In Dunal's original description the type is said to have glabrous or glabrate stems and sub-glabrous peduncles, but he notes the calyx to be pilose. Branched hairs are not mentioned in Dunal's prologue.

It should be noted that Henrickson, early on, (by annotation at LL_TEX) treated **C. coronopus** as consisting of two intergrading taxa: var. *arida* and var. *coronopus*; Averett (1973) treated these as but elements of a widespread variable **C. coronopus**; Averett (2005) subsequently removed **C. darcyi** from the complex, a taxon of north-central Texas and Oklahoma, but treated **C. arida** within his concept of **C. coronopus**, noting that "I see little justification for the recognition of *C. arida* as a separate species but, with further analyses, it plausibly might be recognized at the varietal level." With the exclusion of **C. arida** (as treated herein) and **C. darcyi**, **C. coronopus** is largely restricted to southern Texas and closely adjacent Mexico.

In southern Texas the occasional hypothetical hybrid between **C. coniodes** and **C. coronopus** may be found, the two taxa being sympatric throughout most of the region.

CHAMAESARACHA CRENATA Rydb., Mem. Torrey Bot. Club 4: 368. 1896. **Maps 4a, 4b**

TYPE: **MEXICO. COAHUILA: Mpio. Parras De La Fuente,** Jun 1880, *Palmer 923* (holotype US!; isotypes GH!, K!, NY!,).

Chamaesaracha villosa Rydb., Mem. Torrey Bot. Club 4: 368. 1896.
TYPE: **MEXICO. COAHUILA: Mpio. Escobedo**, 24 mi NE of Monclova, Sep 1880, *Palmer 924* (holotype US!; isotypes GH!, K!, NY!).

Suffruticose, perennial, herbs 10-30 cm high. Stems more or less fractiflex, arising from a large ligneous tap-root, moderately to densely pubescent with simple or elongate dendritic trichomes 2-3 mm long, under this a moderate to sparse display of very short glandular hairs, less often with mostly short glandular hairs. Leaves alternate, gradually reduced upwards, those at mid-stem mostly 3.5-7.0 cm long, 2.5-4.5 cm wide; petioles 1-2 cm long, rather abruptly grading into the blades; blades ovate, pubescent like the stems, irregularly crenate to nearly entire. Flowers 1-3 on peduncles mostly 1-3 cm long. Calyx 3.5-5.0 mm long,

densely pubescent like the stems. Corollas rotate, 1.0-2.5 cm across, pale yellow to yellow. Stamens exserted for ca 4-5 mm; anthers yellow. Style exserted ca 5.5 mm. Capsule orbicular, ca 7 mm across. Seeds ca 2 mm across, adorned with an irregular reticulation of raised ridges. Chromosome number, 2n = 12 pairs.

Averett (1973) recognized *C. villosa* and *C. crenata* as distinct species, but acknowledged that they were closely related and that

> The two species are largely allopatric, but their ranges overlap in Coahuila and in Trans-Pecos, Texas (Fig. 9). In the latter area, particularly in Big Bend National Park and around Lajitas (Brewster and Presidio Co.), the two species are often difficult to distinguish and hybridization is suspected [and occasional sheets are annotated as such, e.g. *Averett & Sikes 237*]. At the extremities of their ranges, however, the two species are fairly clear and they can be distinguished by the characters given in the key to species.

I agreed with Averett's assessment early on, but additional collections and fieldwork in Mexico suggest the overlap of the two is considerable and difficult to distinguish. Indeed, collections from Mpio. Parras, Coahuila, the type area of **C. crenata,** are as "villose," or more so, than *C. villosa* itself. In short, I now believe that the recognition of a single, highly variable, robust species is the preferred treatment. This assessment agrees with that of Henrickson (2009) who combined *C. villosa* and **C. crenata**, taking up the former name for the "inclusive species," for reasons unspecified, both published at the same time. In my opinion the proper name for the duo, if synonymized, should be **C. crenata**, its author publishing *C. villosa* as an afterthought, or footnote to the former, this on the same page.

Averett (2010a), however, retained both taxa. After reviewing again their taxonomic history, he summed up the matter, as follows: "since the two taxa are distinct in and around their type localities and in all but a few populations in Trans-Pecos, Texas, I believe the continued recognition of *C. crenata* and *C. villosa* is warranted." This logic I follow not, for the types concerned are quite similar in southern Coahuila, Mexico, although most collections from Mpio. Parras (from whence the type of **C. crenata**) are more densely villous than those of the reputed *C. villosa.*

Chamaesaracha crenata (including *C. villosa*) is largely confined to the Trans-Pecos, Texas and north central Mexico. With additional collections, especially from Mexico, and rethinking the status of *C. villosa*, my distribution of the species differs somewhat from that pictured by Averett for both **C. crenata** and *C. villosa.* In particular, I believe that some of the south Texas sites of **C. crenata** represent occasional robust plants of **C. coniodes**, which also is frequently difficult to distinguish without a chromosome count.

Some further discussion of *Chamaesracha villosa* may be warranted. The species is typified by material collected northeast of Monclova, Coahuila, and is characterized by its relatively robust habit, large leaves and a dense vestiture of very long, mostly flagelliform forked or branched trichomes. Beneath this upper story of elongate hairs there occurs a much shorter under-story of short glandular trichomes, sometimes dense, sometimes sparse. Scudday (1965) first noted such populations in Presidio Co., Texas that he compared to *C. villosa*; similar variation occurs only along the Rio Grande River in western Texas; I view all such collections as **C. crenata**.

CHAMAESARACHA DARCYI Averett, Monogr. Syst. Bot. Missouri Bot. Gard. 104: 350. 2005.
Map 5

TYPE: **USA. TEXAS: Palo Pinto Co.:** "At campground area, Lake Possum Kingdom on Hwy 36. Red sandy soil," 27 Jun 1969, *Averett & Bierner 474* (TEX).

Averett, with his original description, gave an excellent account of the taxon, and such need not be expounded upon here except to note his taxonomic appraisal:

> [**Chamaesaracha darcyi**] is an eastern group of populations largely restricted to the Rolling Plains of north-central Texas and adjacent Oklahoma. The species is very close to *C. coniodes*, having a dense vestiture of branched, dendritic hairs like those found on the type of *C. coniodes*. However, *C. darcyi* typically has more deeply lobed or toothed leaf margins and a nearly prostrate habit. The species is also disjunct from populations in south Texas with a similar vestiture and east of populations with unbranched simple trichomes.

CHAMAESARCHA EDWARDSIANA Averett, Sida 5: 48. 1972. **Map 6**

TYPE: **USA. TEXAS: Travis Co.:** 0.5 mi. east of the Pedernales River along highway 620, 15 Jul 1968. *Averett 289* (holotype TEX!; isotypes MO, SMU, US).

This species and its estimated parameters as treated by Averett need no revision, except for its exclusion from Mexico, as noted below. It is closely related to **C. pallida** but can be distinguished from it by having peduncles with predominantly straight glandular hairs (vs. dendritic, eglandular hairs), mostly narrower leaves and by distribution. Occasional plants will display along their peduncles an admixture of both glandular and eglandular dendritic hairs (e.g., Blanco Co., *Watson & Averett 179* (TEX); Concho Co., *Averett 358, 485* (TEX); Crockett Co., *Turner 99-30* (TEX); Kinney Co., *Strother 253* (TEX); Menard Co., *Turner 21-784* (TEX); Schleicher Co., *Turner 98-496* (TEX); Val Verde Co., *Webster 32284* (TEX); I have treated these as hypothetical intermediates.

As noted in the introduction to this paper, pubescence may be under relatively simple genetic control that might account for the variability in vestiture observed among and between populations of **C. coronopus**. Alternatively, occasional hybridization between **C. edwardsiana** and **C. coronopus** may confound the distinctions between these and **C. pallida**, the latter possessing a vestiture resembling that of **C. coronopus**, but having habital features and leaf margins of C. **edwardsiana**.

Vestiture in **C. pallida** varies in similar fashion to that of **C. edwardsiana**, occasional plants of the former will have peduncles mostly without glandular hairs; nevertheless occasionally populations of **C. pallida** (e.g., Brewster Co., *Warnock T283*, TEX) will have plants with glandular peduncles, approaching those of **C. edwardsiana**, but otherwise typical.

Finally, it should be noted that Averett, with his original description, maps ten or more collections of **C. edwardsiana** as occurring in the states of Coahuila and Nuevo Leon, Mexico; I take nearly all such collections to be specimens of **C. pallida,** and these will key as such in the present treatment.

CHAMAESARACHA FELGERI B.L. Turner, **sp. nov. Map 7**

TYPE: **U.S.A. ARIZONA: Yuma Co.,** Barry M. Goldwater Military Range, Coyote "Wash" at Camino del Diablo, bottom of Lechugilla Valley, SE of Tinajas Atlas, 1010 ft, "Lowest point on Camino crossing, there is a sheet flow here in the valley bottom but no wash (erosion) has formed." 25 Oct 2004, *Richard C. Felger 04-63* (Holotype: TEX; isotypes: ARIZ, ASU).

Perennial herbs 20-30 cm high, arising from deep seated roots. Stems pubescent with elongate simple or dendritic trichomes 2-3 mm high, beneath these a uniform layer of short, branched trichomes 0.1-0.3 mm high. Mid-stem leaves mostly (7)8-12 cm long, 1-2 cm wide, moderately to sparsely pubescent with stellate hairs (not cottony pubescent), the margins entire (lowermost) to markedly lobate. Flowers one or two to a node, the pedicels mostly 3-4 cm long, pubescent like the stems. Calyces (flowering), 5-6 mm

long, with lobes 2-3 mm. Corollas (flared) 2.5-3.0 cm across, reportedly "cream-yellow," pale yellow," or whitish." Mature fruit not observed.

This novelty resembles **C. arida**, but differs in having larger mid-stem leaves (ca 7-12 cm long vs 3-5 cm), longer hairs on both stems and calyx (ca 2-3 mm long vs 0.5-1.0 mm) and larger, more expanded, corollas (2-3 cm across vs 1-2 cm).

ADDITIONAL COLLECTIONS EXAMINED: **USA. ARIZONA: Yuma Co.,** "Pinta sands along Camino del Diablo, Cabeza Prieta National Wildlife Refuge." 780 ft, "Low dunes with extensive populations of spring ephemerals. Common; perennial from deeply buried roots, the plants often buried in sand," West Pinta Sands. 780 feet. Low stabilized dunes." (32 08 N, 113 33 W) 1 Feb 1992, *Felger 92-626* (TEX); near same locality, 16 Jun 1992, *Felger 92-626* (TEX). Cabeza Prieta National Wildlife Refuge, Camino del Diablo, at 2 mi E of western edge of lava flow (32 06 56 N, 113 31 20 W), 28 Nov 2001, *Felger 01-548* (TEX); **ca** same locality, "low, stabilized dunes," 780 ft, 6 Jun 1992, *Felger 92-262* (TEX). [The latter collection is obviously a depauperate specimen, w/o flowers or fruit, collected out of season.]

The elongate dendritic hairs on the calyces, large leaves and large flowers readily distinguish this species from yet other taxa of **Chamaesaracha.** The novelty appears closely related to the smaller flowered, more eastern, **C. arida;** indeed, two plants from the type locality (cited below) were annotated as such by Henrickson. **Chamaesaracha felgeri** is apparently adapted to deep sandy soils and appears restricted to southern Yuma Co. (and perhaps closely adjacent Mexico). Some workers might wish to recognize this as but an edaphic ecotype of the widespread **C. arida**, this suggested by *Felger 92-626*, which approaches that taxon in pubescence; indeed, Henrickson annotated the latter collection and *Felger 92-26* as *C. coronopus* var. *arida* [now **C. arida**]. I concede that it is possible that the two taxa might come into contact in Yuma Co. and form the occasional hybrid, although I have not examined plants of the latter from the area concerned. Clearly, both taxa are in need of additional field study.

The species is named for the well-known southwestern collector, Richard Felger, who participated in the collection of all the specimens of the taxon known to me.

CHAMAESARACHA GEOHINTONII Averett & B.L. Turner, Phytologia 92: 435. 2010. **Map 7**

TYPE: **MEXICO. NUEVO LEON: Mpio. Mina**, "West of Los Molina," gypsum hillside, ca 26 04 N, 100 45 W, 943 m, 23 Jun 2007, *Hinton et al. 28619* (Holotype: LL-TEX).

Perennial herbs, 10-20 cm high. Stems slender, presumably from slender rhizomes, densely pubescent with simple glandular-trichomes 0.5-1.5 mm high. Midstem leaves mostly 1.5-3.5 cm long, 1.5-2.0 cm wide; pedicels 1.5-2.5 cm long, pubescent like the stems. Calyces 3-5 mm long, densely pubescent, the lobes broadly lanceolate. Corollas rotate, greenish-yellow, ca 12 mm across. Stamens 5, 3-5 mm long; anthers yellow, ca. 1.5 mm long. Capsules globular, ca 8 mm across. Seeds, brown, tuberculate, ca 22 to a capsule, 2.0-2.2 mm long, 1.5-2.0 mm wide.

According to Averett (2010), *C. geohintonii* is a gypsophilic species, known only by its type, but closely related to **C. crenata** and **C. villosa**.

This taxon is not easily placed among the described species of **Chamaesaracha**. Averett (pers. comm.) favored a relationship to **C. sordida**, while I favored a position near or within **C. crenata**. Regardless, its isolated geographic position and restriction to gypseous soils strongly suggests novel status. The collector describes the plant as occurring in colonies, suggesting a rhizomatous habit. He also describes the fresh flowers as "green," but in the dry state, on herbarium sheets, these appear greenish-

yellow.

George B. Hinton, from whence the eponym, is the son of the late Jaime Hinton, and grandson of the renown Mexican collector, George B. Hinton, is the only person to have garnered the present novelty, collecting this from an isolated outcrop of gypsum, well known for harboring a number of edaphic endemics (Turner 2008, and citations therein).

CHAMAESARACHA PALLIDA Averett, Sida 5: 49. 1972. **Maps 8a, 8b**

TYPE: **USA. TEXAS: Presidio Co.,** 35 mi SW of Marfa on Pinto Canyon Road, 15 Jul 1966, *Averett 155* (Holotype: TEX; isotypes: GH, MO, SMU, US).

This species is very closely related to **C. edwardsiana**, and might be treated under the latter as but varietally distinct (as sheets so annotated by Henrickson at TEX). I believe, however, the two are distinct, their differences equal to those separating other species within **Chamaesaracha**. The characters used in the key to distinguish between these suggest that typical **C. edwardsiana** is confined to the Edwards Plateau region of central Texas, while **C. pallida** is largely confined to the Trans-Pecos, Texas, New Mexico, and north-central Mexico. Averett (1973) used leaf shape and pubescence to distinguish between the two taxa; however, leaf shape and pubescence is very variable in this alliance and reliance upon such characters probably accounts for the overlapping distribution of these two taxa as depicted in Averett's Fig. 6. It is likely that additional collecting in the easternmost part of the distribution of **C. pallida** will show that intergrades commonly occur between the two. Early on, I tentatively identified several likely intermediates between these as occurring in Sterling Co., Texas (*Averett 309*, TEX) and Irion Co. (*Warnock 7700*, TEX) counties, the plants concerned having nearly entire leaves and a peduncular pubescence like that of C**. pallida**; such plants were subsequently accepted as belonging to the latter, having most of its characters, except for leaf shape. Indeed, nearly entire leaves occur sporadically in most species of **Chamaesaracha**, and reliance upon this character alone is likely to result in the occasional misidentification.

Variation in **C. pallida** is undoubtedly confounded by the occasional hybridization between this and **C. sordida**, as attested to by three specimens assembled on the same sheet (Brewster Co., Glass Mts., *Warnock 21201*, TEX): one of these **C. sordida**, the other two hypothetical hybrids or backcrosses with **C. pallida** (Averett, however, annotated the three specimens as distinct species: **C. coniodes, C. pallida** and **C. sordida**).

Finally, it should be noted that a case might be made for the recognition of a glabrate variety of **C. pallida**, populations of which occur in western Trans-Pecos, Texas. Both "forms," however, occur in the Guadalupe Mts. of Culberson Co. and elsewhere. Averett was aware of these populations but, at the time, there were only two collections and few have been collected since. Future workers need be aware of such collections. Additional comments on the relationships of the above taxa and their intermediates are discussed under **C. edwardsiana**.

CHAMAESARACHA RZEDOWSKIANA A.T. Hunziker, Contr. Gray Herb. 210: 23. 1980. **Map 7**

TYPE: **MEXICO. SAN LUIS POTOSI: Mpio. Xilitla**, Las Cruces, 600 m, 1 Mar 1959, *Rzedowski 10103* (holotype GH).

This is a very distinct species of **Chamaesaracha,** what with its creeping habit and markedly fractiflex stems; the novelty was known to its author only by type material. I have examined three additional collections, as follows: **QUERETARO. Mpio. Jalpan,** Los Sarros, 50 m, 30 Mar 1993, *Lopez Ch. 546* (TEX); about Tanchanaquito, 450 m, 11 Mar 1993, *E. Carranza et al. 4580* (TEX). **SAN LUIS**

POTISI. Mpio. Aquismon, Mante Tsuled, 27 May 1979, *Alcorn 3065* (TEX). The latter collection is remarkably delicate, with less pubescent foliage and smaller flowers, superficially resembling a species of the genus **Dichondra**. Nevertheless, it has all of the characteristics of **C. rzedowskiana** and would appear to be but an impoverished or perhaps shade form of that species. Hunziker (1980), with his original description, provided an excellent illustration of the species.

There is little material of this species in herbaria and its relationship to other species of **Chamaesaracha** seems obscure. It differs in habit and basic leaf morphology from yet other species of the genus and its range represents a significant disjunction from other elements of the genus.

CHAMAESARACHA SORDIDA (Dunal) A. Gray, Syn. Fl. N. Amer. 1: 232. 1876. **Maps 9a, 9b**
Withania sordida Dunal in DC., Prod. 13: 456. 1852.

TYPE: **USA. TEXAS: Webb Co.,** near Laredo, Aug 1829, *Berlandier exs. 2076* (holotype: G-DC, microfiche TEX!; isotypes: GH, NY).

My concept of **C. sordida** is about the same as that of Averett (1973), and this need not be described anew. The species is partially sympatric with **C. conoides, C. darcyii, C. arida,** and **C. coronopus** and is readily distinguished from these and others by its rather uniform array of short glandular trichomes, the latter always predominating over any display of branched hairs. Branched hairs, however, are not uncommonly found in **C. sordida**, a smidgen of which intermixed with a much more numerous display of glandular hairs occasional occur in this or that population of yet other taxa.

It is possible that such admixtures are due to the occasional hybridization of **C. sordida** with one or more of the other species, but the great mass of the specimens, as shown in Map 6, possess mostly short glandular hairs, but eglandular trichomes are relatively common. Further evidence of hybridization is suggested by chromosome counts. Averett (1973) noted three widely separated triploid populations with chromosome numbers of n = 18 (all other counts are n = 12) which probably result from crosses with tetraploid (n = 24) populations. There was, however, no morphological evidence that another taxon had contributed to these hybrids.

EXCLUDED SPECIES

Chamaesaracha cernua (Donnell Smith) A. T. Hunziker, Contr. Gray Herb. 210: 23. 1980.
Basionym: *Athenaea cernua* Donnell Smith, Bot. Gaz. 48: 297. 1909. [GUATEMALA, Dept. Alta Verapaz, Sasia, 900 m, May 1908, *Tuerckheim II.2245*] Type (US!).

Chamaesaracha potosina Rob. & Greenm., Amer. J. Sci. 50: 161. 1895.
Saracha potosina (Rob. & Greenm.) Averett, Ann. Missouri Bot. Gard. 57: 380-382. 1971 [Type: Mexico. San Luis Potosi: Tamasopo Canyon. Nov 1880, *Pringle 3654* (VT!]), Isotype (GH!)

Hunziker (2001) treated the above taxa, along with **C. rzedowskiana**, as belonging to **Chamaesaracha** sect. Capsicophysalis, and notes the section might be treated as a separate genus. The species have a calyx that tightly invests the young ovary, but at maturity the large red berry ruptures the calyx, the latter usually reflexed; **it also** seems to lack the red fruit and deflexed calyx.
Chamaesaracha spruceana (Hunz.) Hunz., Lorentzia 8: 8. 1995.
= *Darcyanthus spruceanus* (Hunz.) Hunz., Bol. Soc. Argent. Bot. 35: 345. 2000.
Basionym: *Physalis spruceana* Hunz., Kurtziana 1: 208. 1961.

Hunziker (2001) relegated this species to the monotypic genus *Darcyanthus*. He suggested that it was most closely allied with **Capsicum** and related genera.

Chamaesaracha viscosa (Schrader) Hunz., Lorentzia 8: 8. 1995.
= *Schraderanthus viscosus* (Schrader) Averett, Phytologia 91: 55. 2009.
Basionym: *Saracha viscosa* Schrader, Index Seminum [Goettingen] 5. 1832.
Witheringia viscosa (Schrad.) Miers, Ann. Mag. Nat. Hist., ser. 2, 11: 92. 1853.
Athenaea viscosa (Schrad.) Fernald (Proc. Amer. Acad. Arts 35: 567. 1900.
Jaltomata viscosa (Schrad.) D' Arcy & T. Davis, Ann. Missouri Bot. Gard. 63: 363. 1976 [1977].
Leucophysalis viscosa (Schrad.) Hunz., Kurtziana 21: 283. 1991.

Schraderanthus viscosus, as noted in the synonymy above, has enjoyed a variety of treatments. Averett (2009) placed the species in the monotypic genus **Schraderanthus** and provided a history of its taxonomy.

ACKNOWLEDGEMENTS

My Academic son, John Averett, provided considerable taxonomic input during an early draft of this paper. Jana Kos provided editorial assistance. Distribution maps are largely based upon specimens on file at SRSC and LL-TEX.

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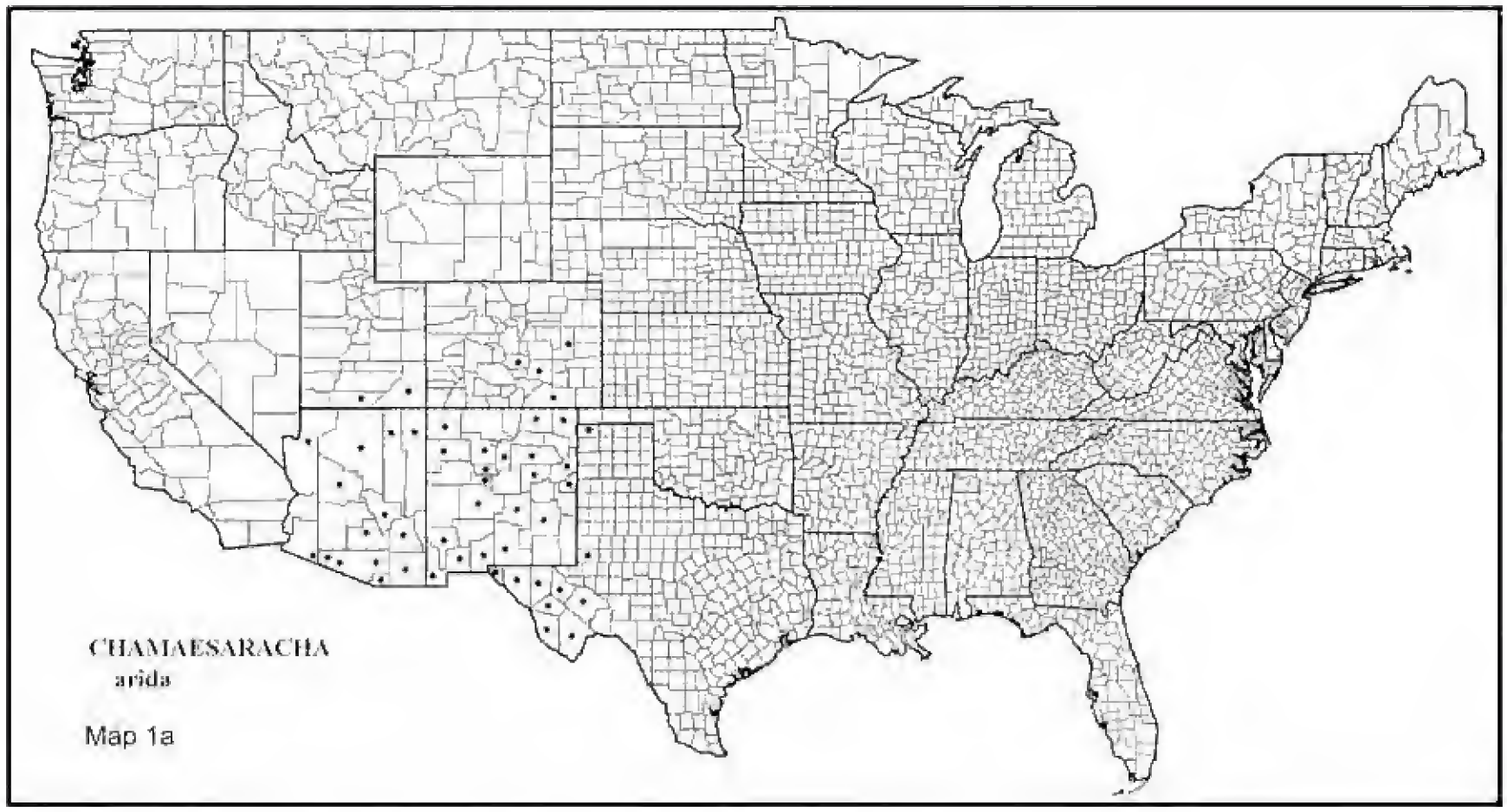
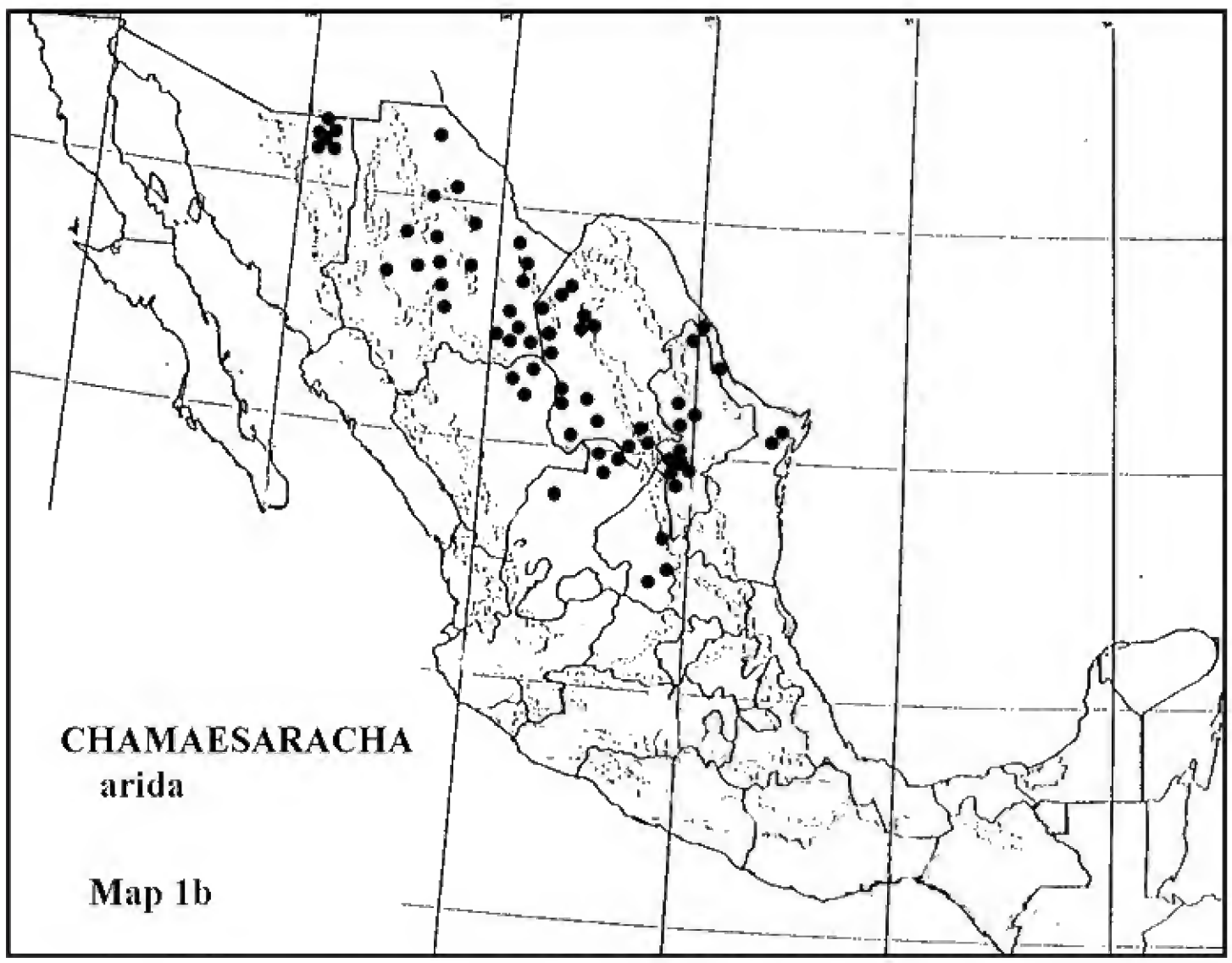
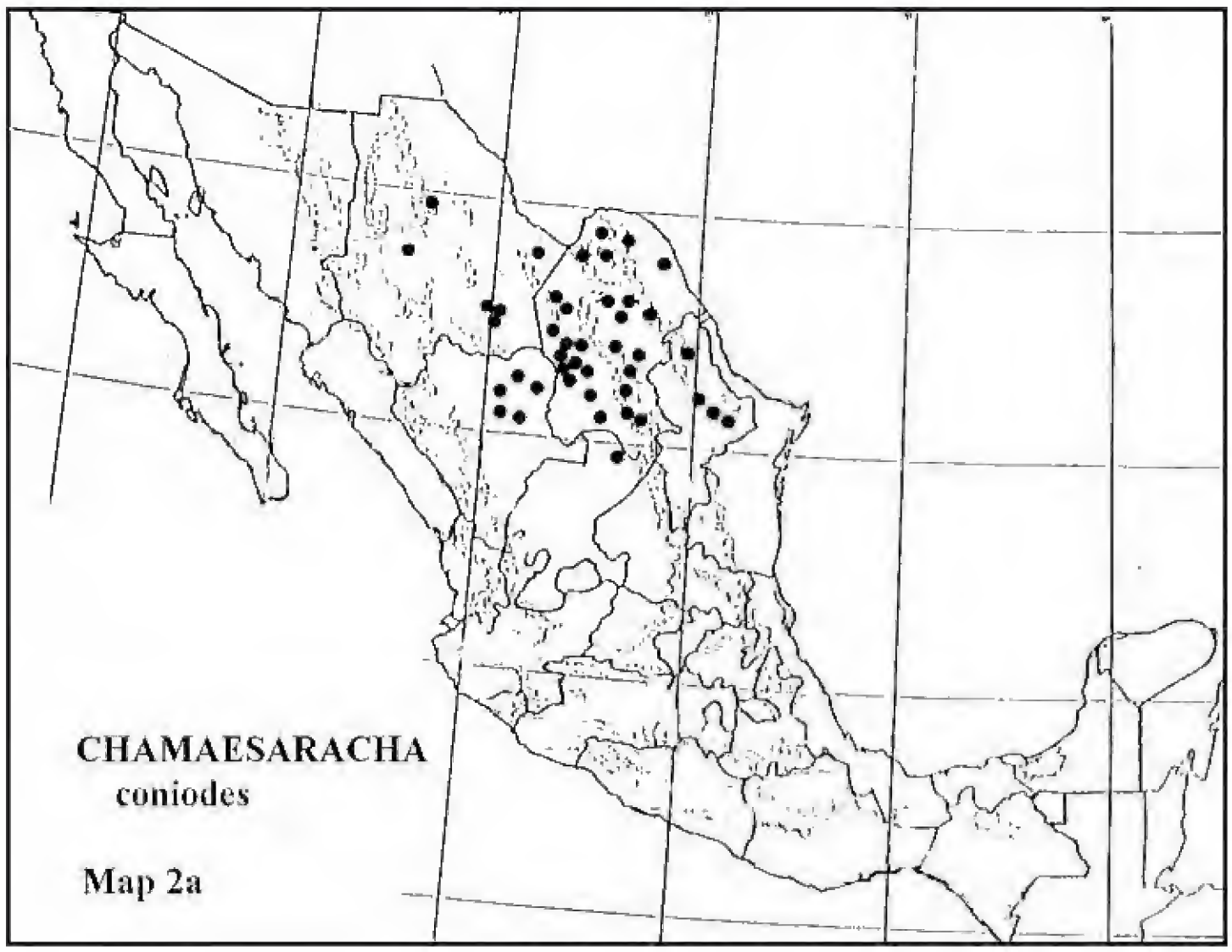
Fig. 1. *Chamaesaracha felgeri* (holotype).



Map 1a

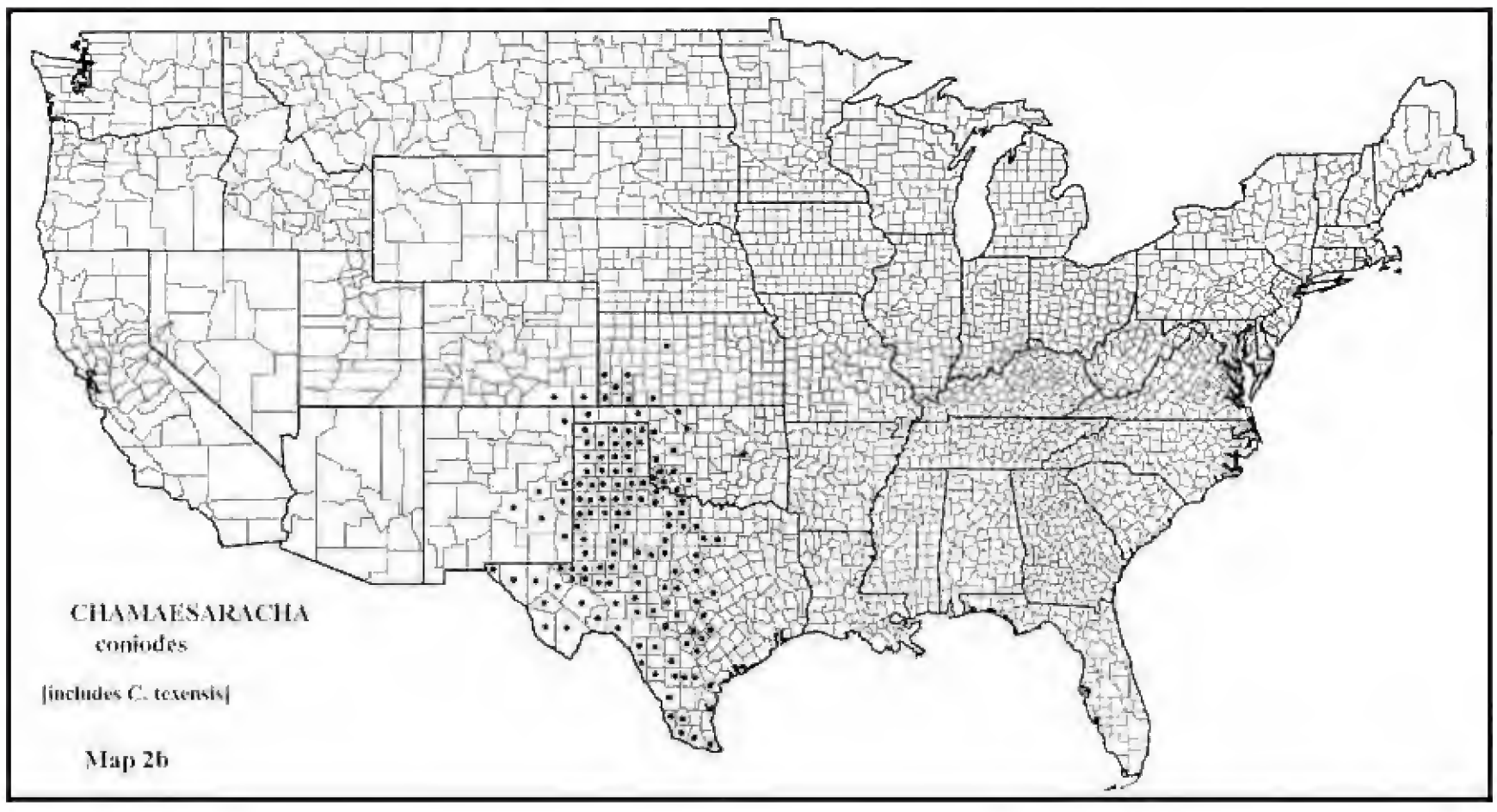


Map 1b



CHAMAESARACHA
coniodes

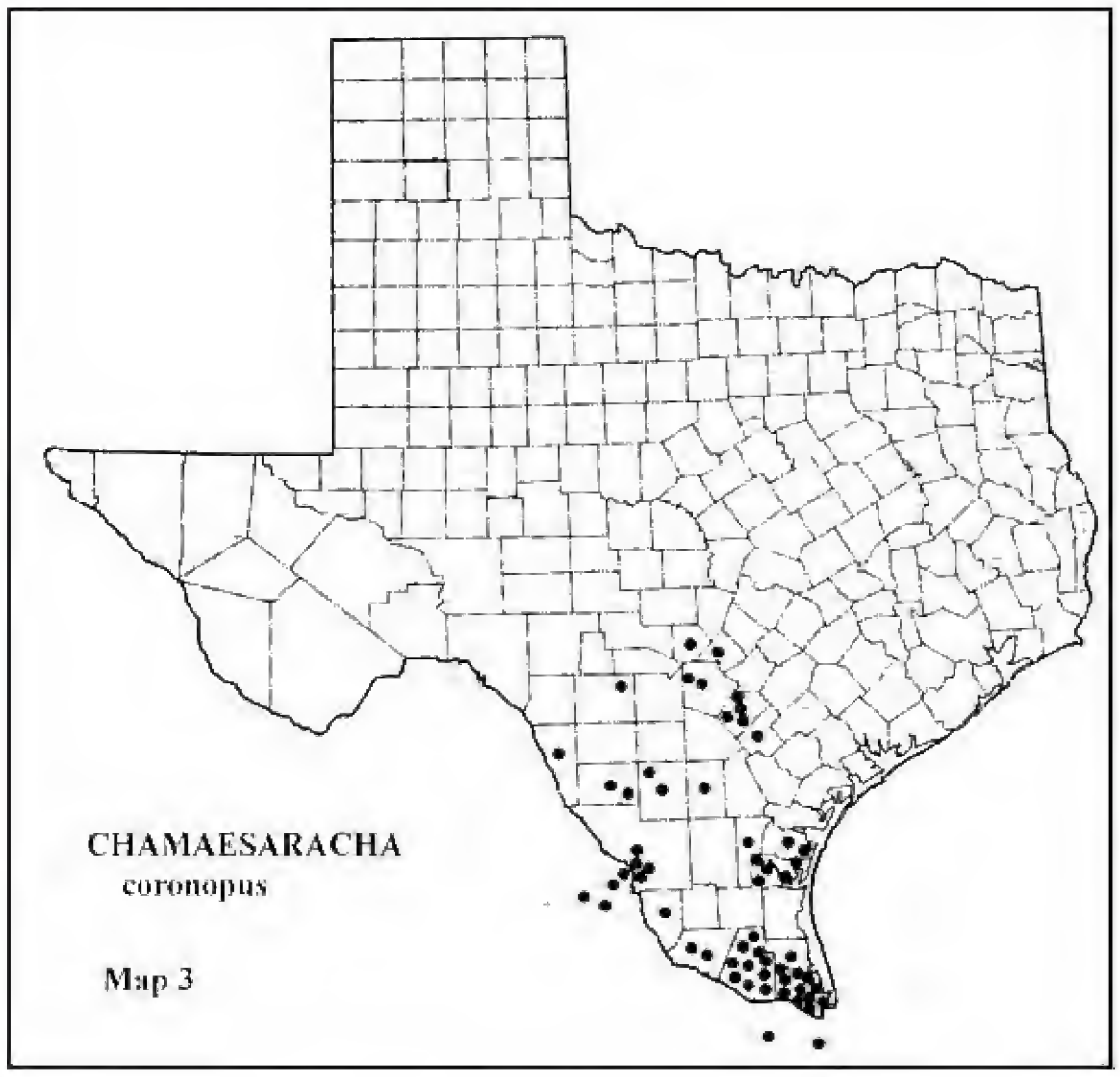
Map 2a

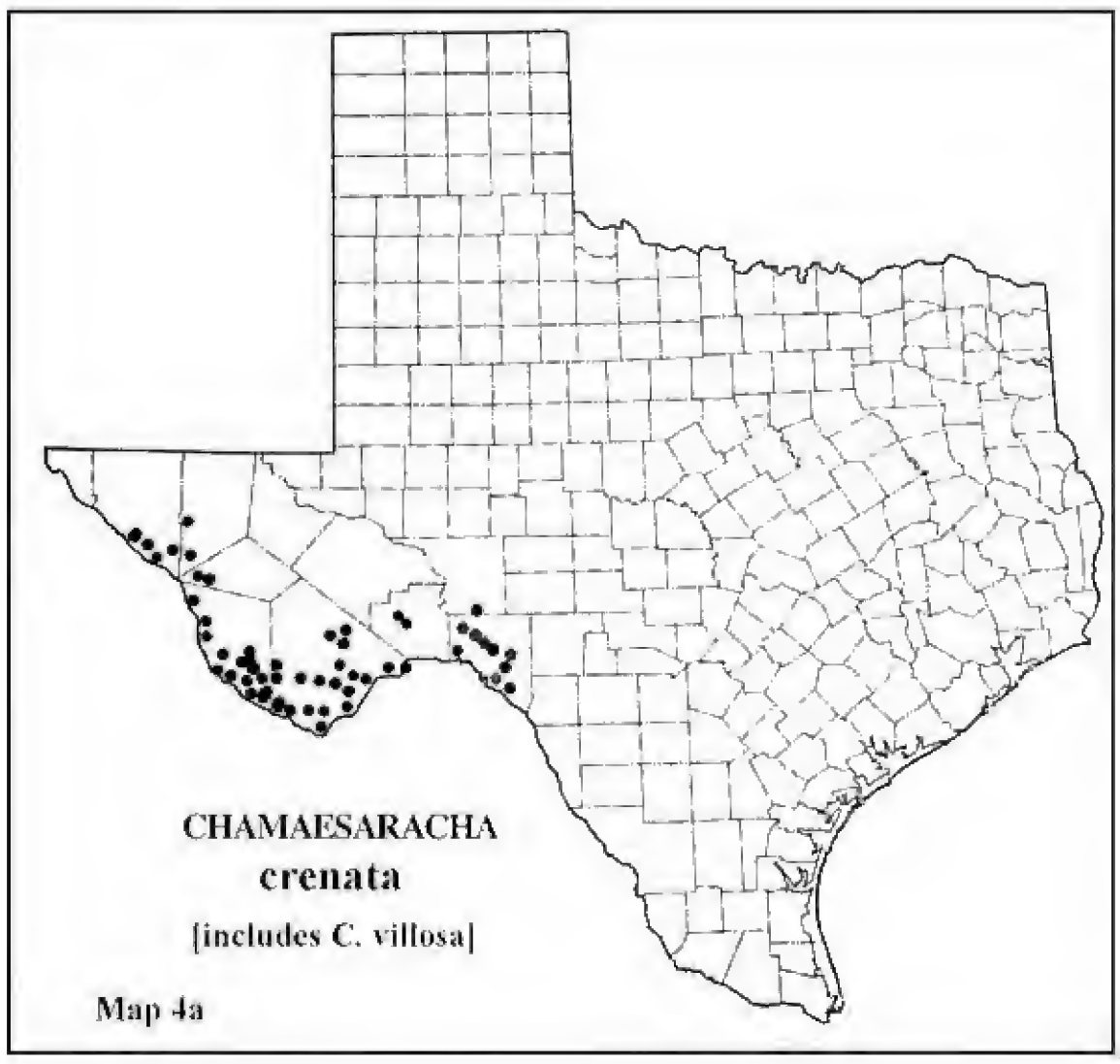


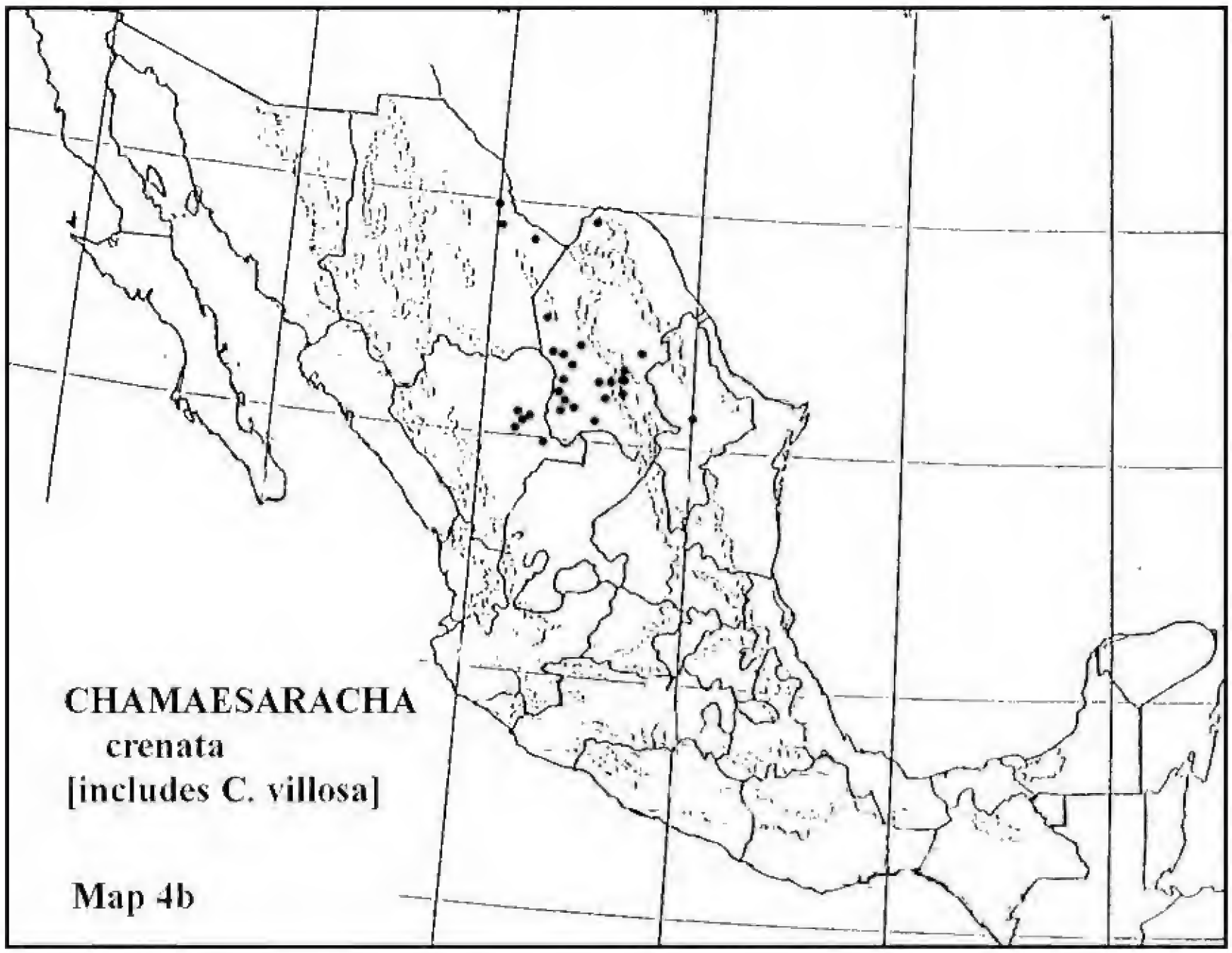
CHAMAESARACHA
coniodes

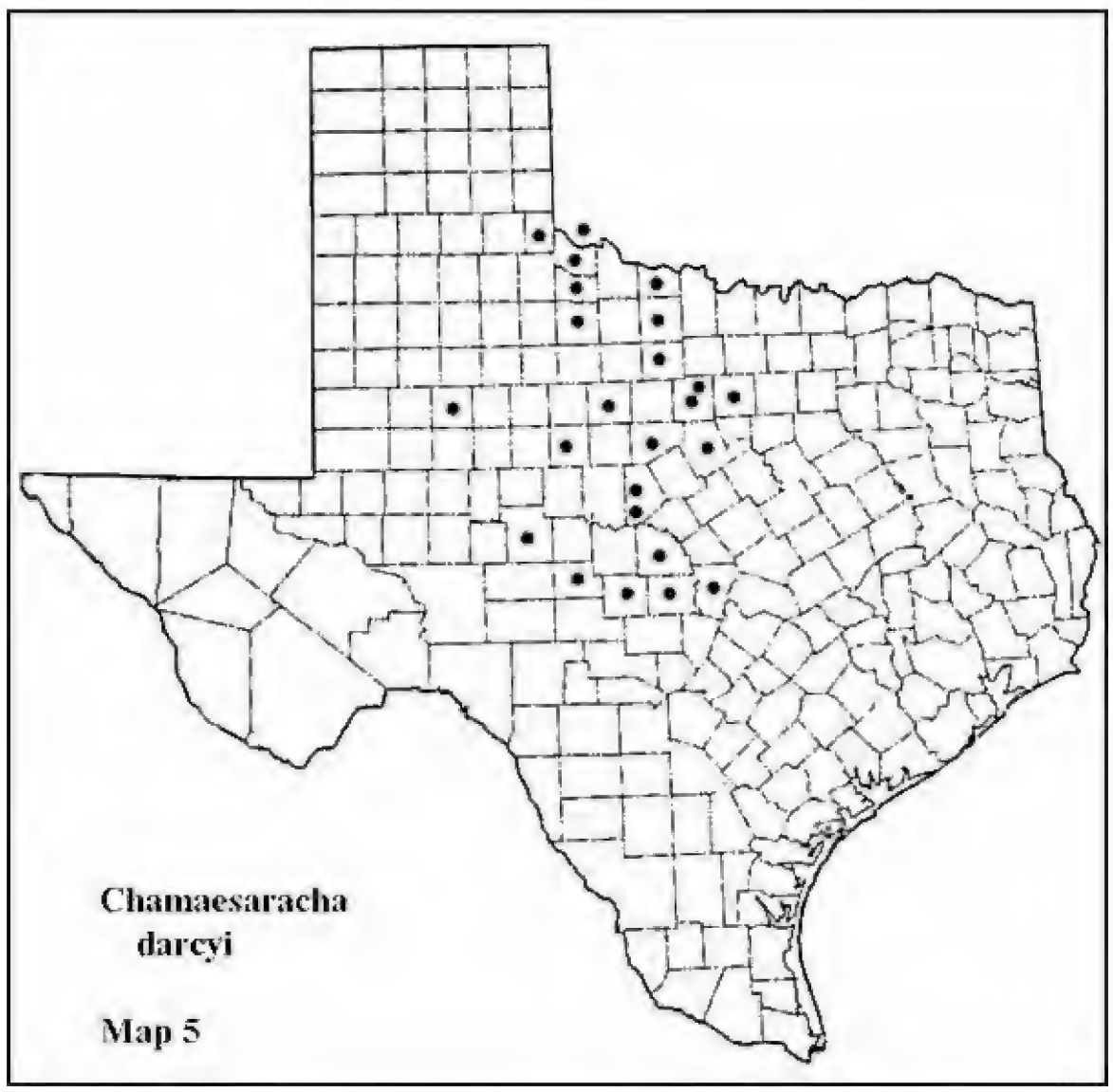
[includes *C. texensis*]

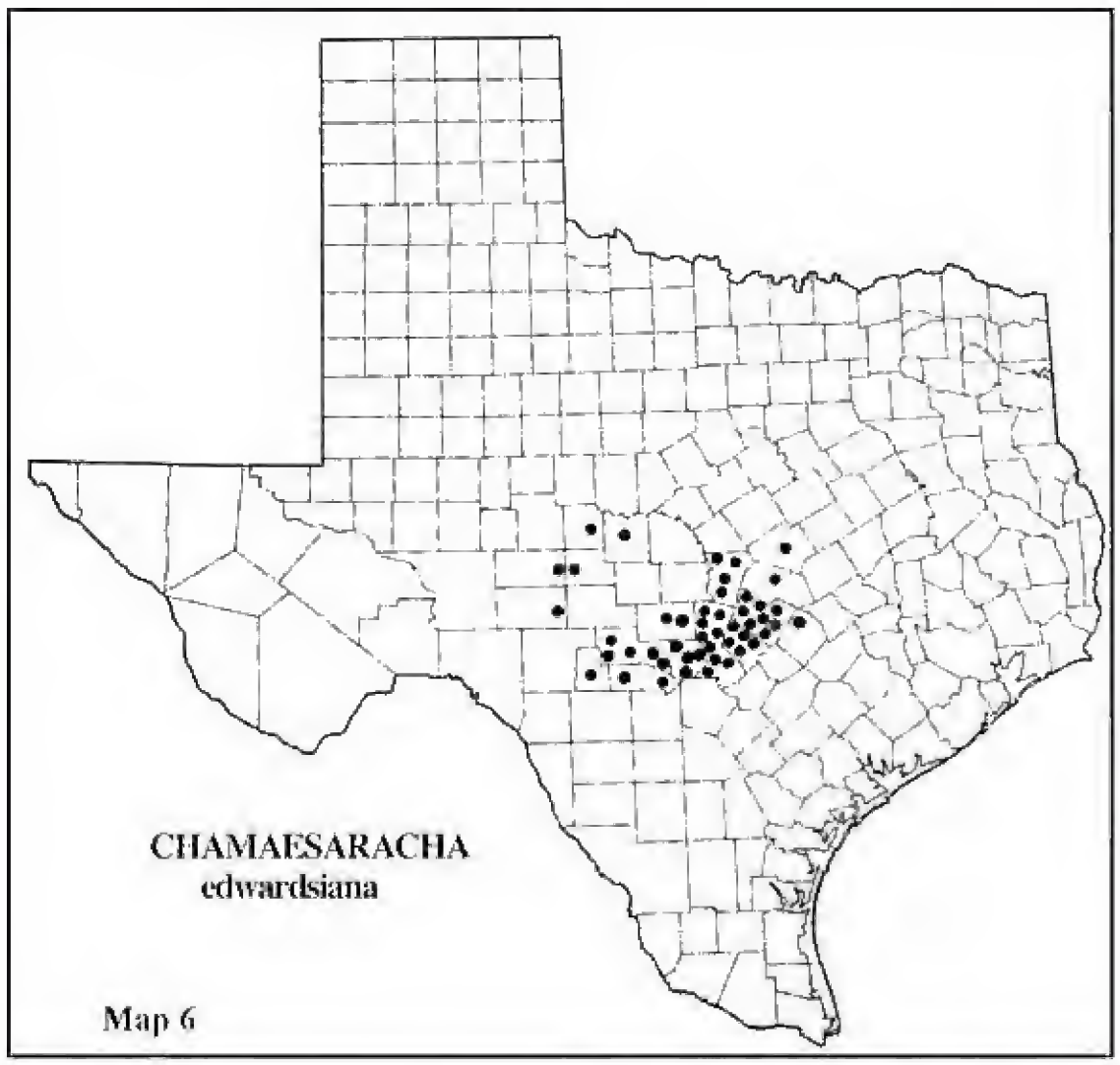
Map 2b


CHAMAESARACHA
coronopus
Map 3

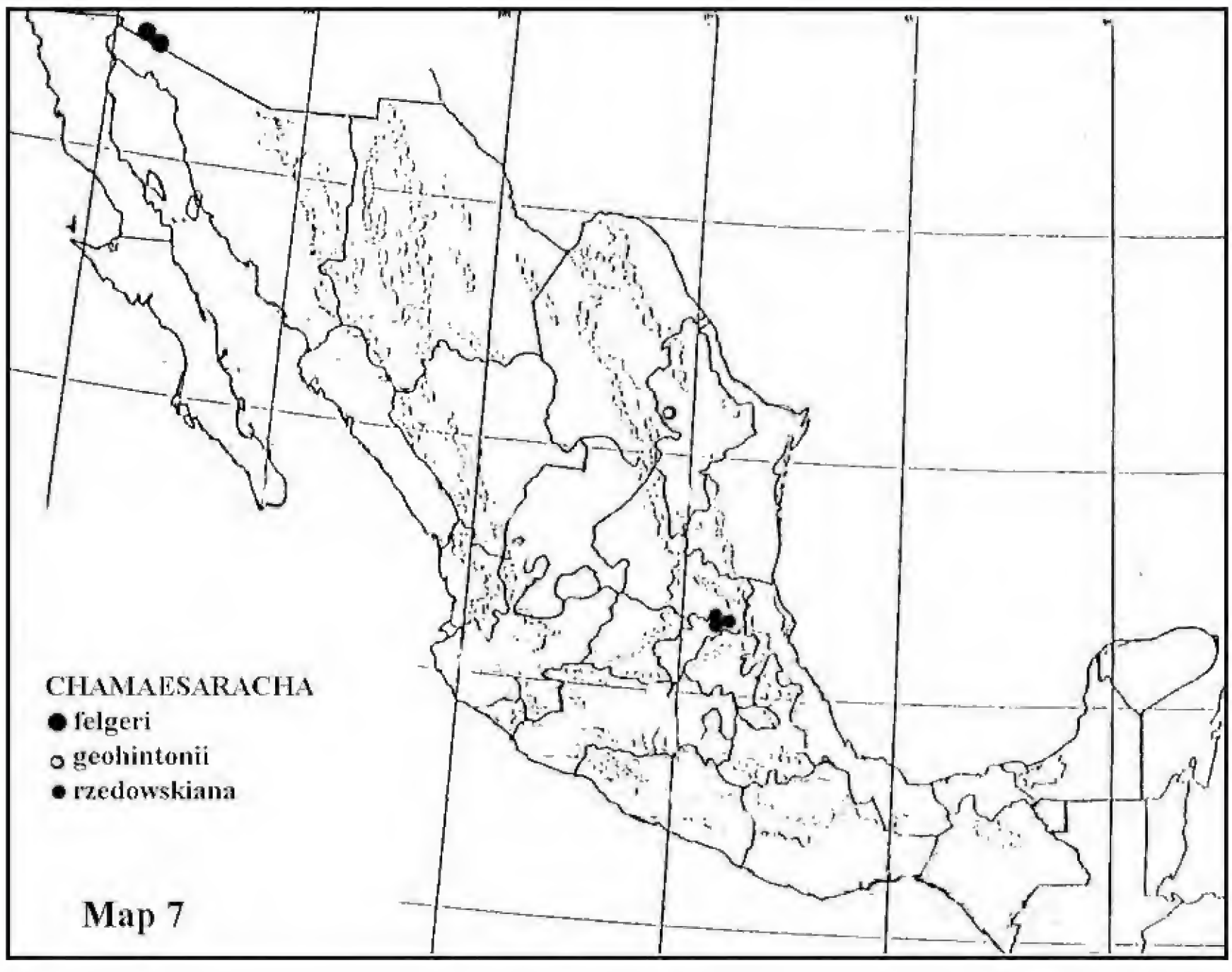

CHAMAESARACHA
crenata
[includes C. villosa]
Map 4a


CHAMAESARACHA
crenata
[includes C. villosa]
Map 4b

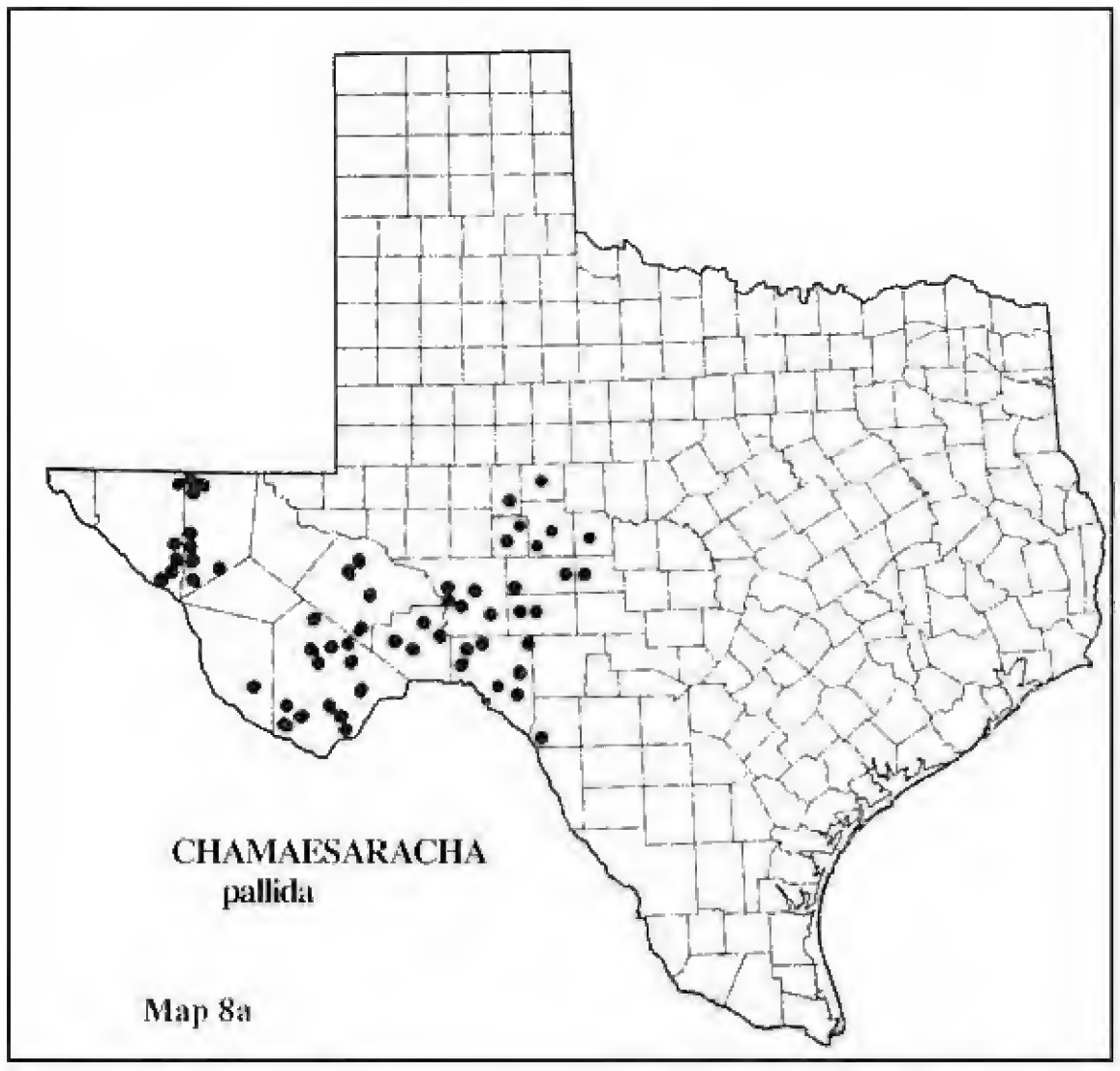

Chamaesaracha
darcyi
Map 5


CHAMAESARACHA
edwardsiana

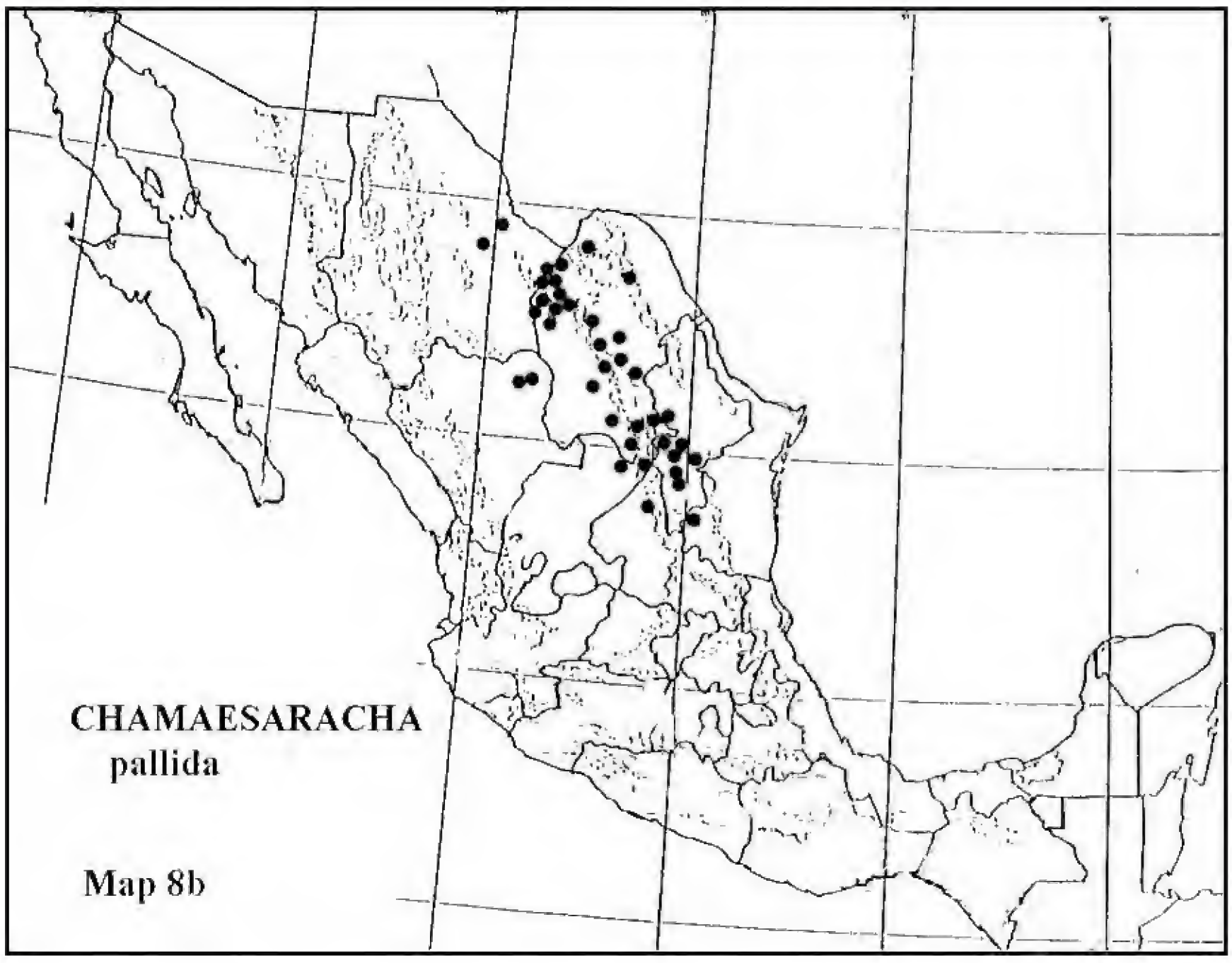
Map 6


CHAMAESARACHA
felgeri
geohintonii
rzedowskiana

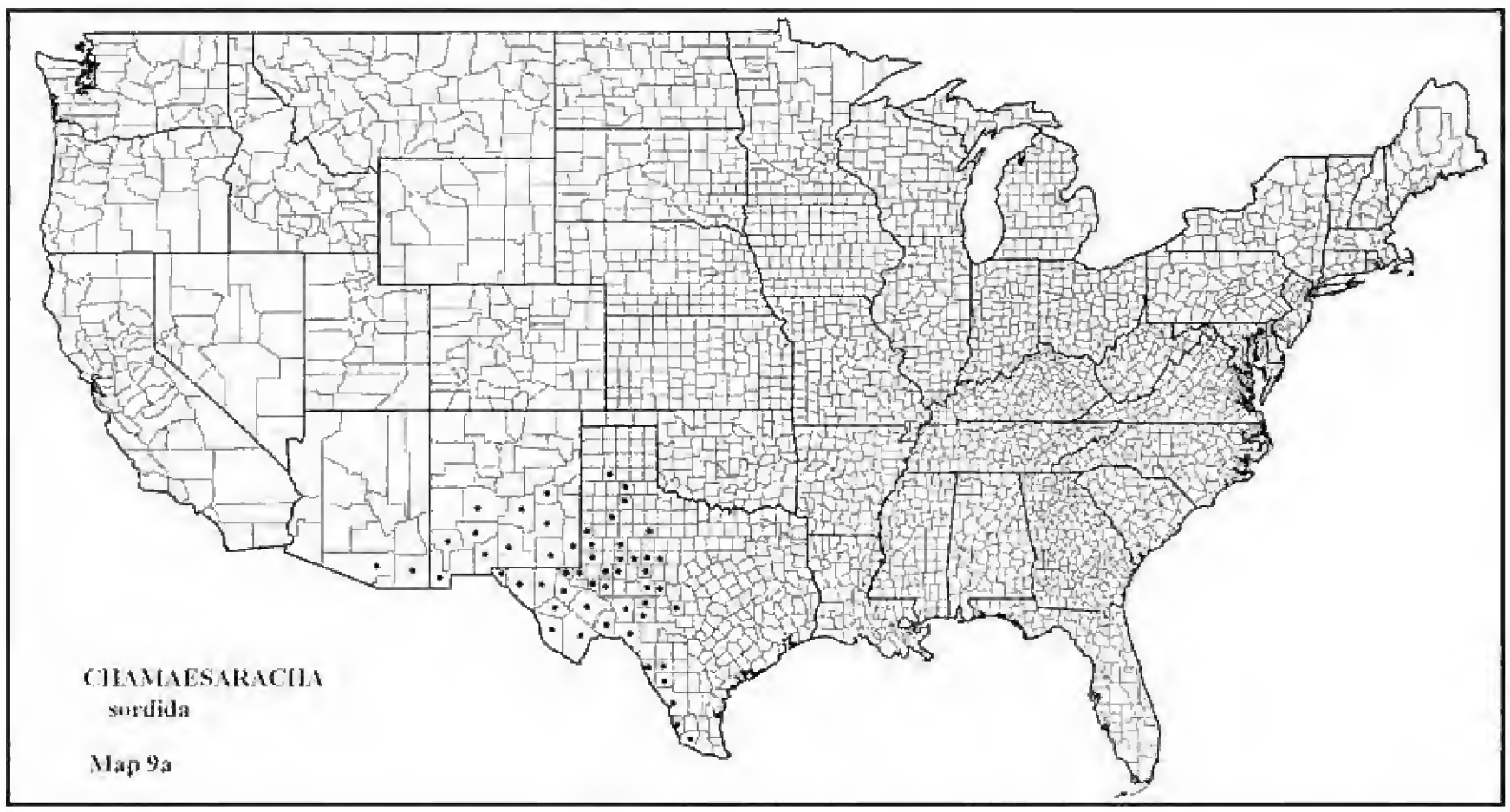
Map 7



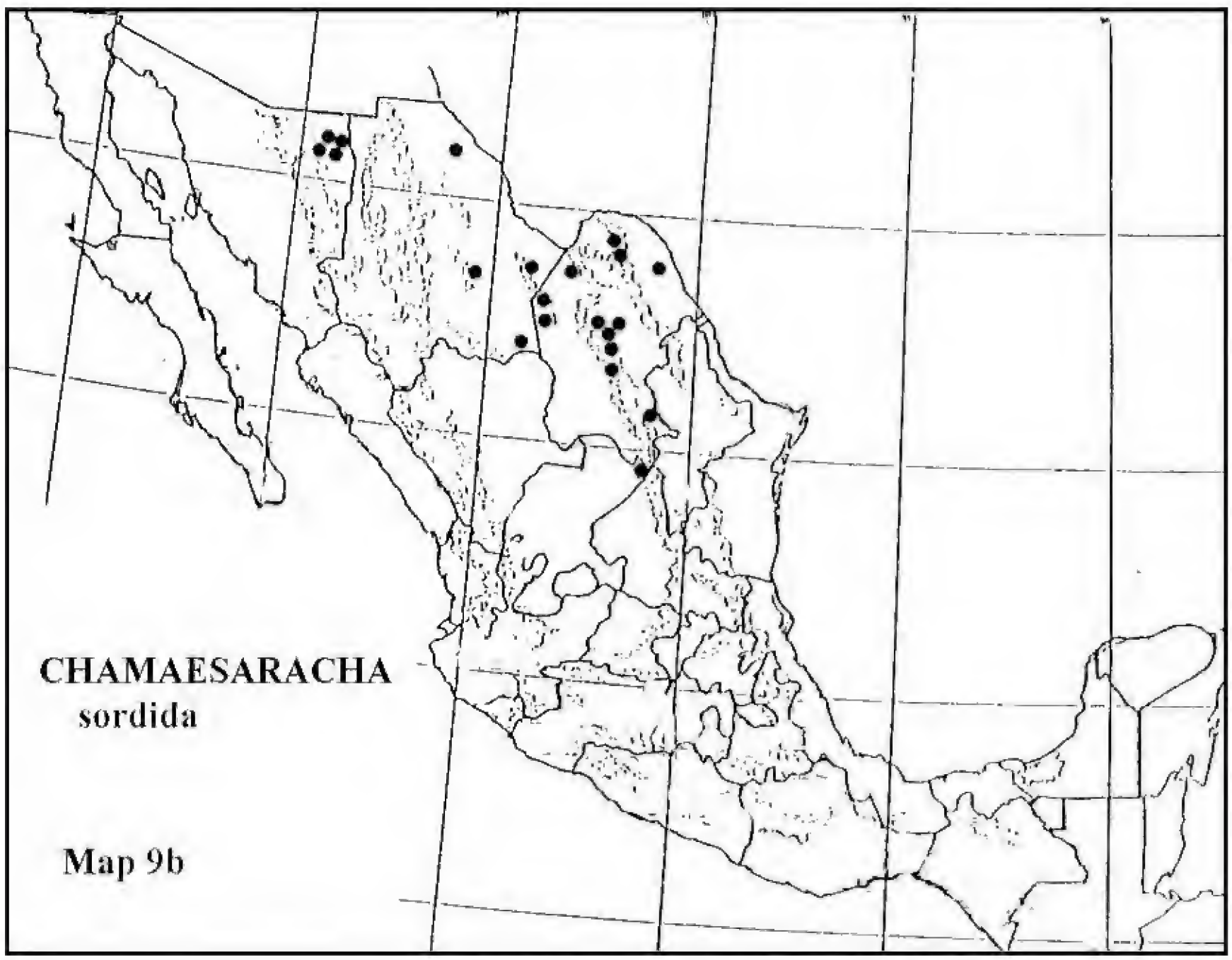
Map 8a



Map 8b



Map 9a



Map 9b

New synonyms and comments on *Phoradendron* (Viscaceae)

Job Kuijt
649 Lost Lake Road, Victoria, BC V9B 6E3, Canada
jobkuijt@uvic.ca

ABSTRACT

Among the new species of *Phoradendron* proposed by Wiens and Hawksworth (2002 [2004]), *P. abietinum* Wiens, *P. flavomarginatum* Wiens, *P. hawksworthii* Wiens, and *P. sedifolium* Wiens are regarded as synonyms of *P. bolleanum* (Seem.) Eichler as circumscribed in Kuijt (2003). *P. acuminatum* Wiens is a later homonym of *P. acuminatum* Kuijt, and is thus illegitimate. *P. rufescens* Wiens becomes a synonym of *P. tetracarpum* Kuijt; *P. olivae* Wiens is similarly referred to *P. minutifolium* Urban, *P. chazaroi* Wiens to *P. dipterum* Eichler, and *P. durangense* Wiens to *P. falcifer* Kuijt**.** An amended description, illustration, and neotype are provided for *P. calvinii* Wiens. Published on-line www.phytologia.org *Phytologia 97(3): 246-251 (July 1, 2015).* ISSN 030319430.

KEY WORDS: *Phoradendron abietinum*, *P. acuminatum*, *P. bolleanum*, *P. calvinii*, *P. chazaroi*, *P. durangense*, *P. flavomarginatum*, *P. hawksworthii*, *P. olivae*, *P. sedifolium*, Viscaceae.

The main purpose of the present contribution is an evaluation of the new species of *Phoradendron* (Viscaceae) that were published in an article (Wiens & Hawksworth 2002[2004][1]) shortly after the appearance of my monograph of the genus (Kuijt 2003). However, I take this opportunity to make a couple of other related comments and to add a new synonym of my own.

The northern reaches of this large, mainly tropical and subtropical genus show a bewildering polymorphy and are therefore difficult to deal with taxonomically. This is true for the leafy species now named *Phoradendron leucocarpum* (Raf.) Rev. & M. C. Johnst. (Abbott & Thompson 2011) as well as for the nearly squamate assemblage of species related to *P. juniperinum* Engelm., and especially for the complex that I united under the name of *P. bolleanum* (Seem.) Engelm. – a complex that geographically reaches from Guatemala to southern Oregon. It is especially in Mexico that the latter alliance produces taxonomic problems, and it is here that most of the new species described by Wiens and Hawksworth are located.

A few general comments on that complex are relevant. The unranked name *Pauciflorae* has been used for it, as a subunit of subgenus *Boreales* Trel. ex Engler, originally including the *P. juniperinum* assemblage as a separate, unranked unit "*Aphyllae*" parallel to the unranked "*Bolleanae*" in which group several of the Wiens & Hawksworth species are nested. However, I have shown (Kuijt 2003) that both "*Boreales*" and "*Pauciflorae*" are unacceptable categories both nomenclaturally and taxonomically. While a great deal of diversity of leaf shapes and sizes is included in my concept of *P. bolleanum*, the male and female inflorescences are essentially uniform throughout, providing the main rationale for the one-species concept. It is also true that there is a virtually unbroken gradient of leaf shapes and sizes. The Wiens and Hawksworth article strongly diverges from my view in this regard; additionally, it also recognizes *P. pauciflorum* Trel. and *P. saltillense* Trel., species that I synonymized under *P. bolleanum.* Two further Trelease names not accounted for by Wiens & Hawksworth but similarly belonging to the

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[1] The heading of Aliso 21(1) reads "2002", but the actual date of publication was 11 February, 2004.

alliance are *P. tequilense* Trel. and *P. guadalupense* Trel., both also being synonymized in my monograph. I should finally point out that the concept of *P. bolleanum* as used by those authors does not agree with its type specimen as seen in Trelease's Fig. 19a (the fragment), as on p. 37 of their paper they speak of leaves being oblong to occasionally sub-oblanceolate, with a rounded to obtuse apex, the blade dorsiventrally flattened. The leaves of the type specimen of *P. bolleanum*, in reality, are very different.

With respect to the synonymies proposed below that refer to elements of the *P. bolleanum* complex, I wish to reiterate from my monograph that I do not deny the possibility that a more detailed study of this baffling polymorphic alliance might not result in a more structured and nuanced taxonomic presentation requiring the revival of some names, perhaps at the subspecific level. Unfortunately, the new species proposed by Wiens & Hawksworth do not contribute to such a resolution.

Other features are often adduced by Wiens and Hawksworth to support their species concepts. Color variation, however, is an unreliable feature, as exposure to the sun and possibly hosts may influence this factor. The appearance and size of the plant, or the length of its internodes, could well be an expression of the host's vigor. In other words: local differences may well exist, but their taxonomic use requires detailed field confirmation. The hosts of most species of the assemblage are mainly Cupressaceae. Wiens and Hawksworth are careful in listing host records where available, but imply limitations that simply have not been demonstrated. For example, the occurrence of "*P. abietinum*" on *Abies durangensis*, as found in " only a few widely scattered localities" might conceivably represent occasional transfers from other nearby hosts. The occurrence on *Abies*, by itself, is not necessarily a reliable indication of taxonomic distinction; such a host has been recorded as far north as northern California (Kuijt 2003). Notwithstanding a coniferous preference, dicotyledonous hosts such as *Mahonia*, *Quercus* and *Umbellularia* have also been documented (Kuijt 2003).

Epiparasitism

Three new species in Wiens and Hawksworth's article are said to be epiparasitic, and some comments on this topic are therefore warranted. The phenomenon of epiparasitism (i.e., parasitism of one species of mistletoe on another) is well documented in Viscaceae, especially in *Dendrophthora* (Kuijt 1961) and *Phoradendron* (Kuijt 2003). The most prominent group of epiparasites is found in some half dozen species centered around *P. dipterum* Eichler. It may well be an exclusive condition in some species, but this fact is difficult to document. In addition to the (perhaps exclusive) epiparasitism in the *P. dipterum* alliance, we occasionally find the phenomenon in species not normally epiparasitic. My view on Wiens and Hawksworth's statements on the collection labels that refer to "Obligate" epiparasitism is that these facts are unproven; all that may be said is that the specimens collected were parasitic on other mistletoes. This is particularly relevant in *P. calvinii* and *P. durangense*; *P. chazaroi* is clearly a small form of the variable and widespread epiparasitic *P. dipterum.*

1. ***Phoradendron abietinum*** Wiens, Aliso 21(1): 37. 2002 [2004], ***syn. nov.***

Wiens et al. 5230, holotype US!; isotype RSA!, MEXU!, MO! Coll. 1975.
Paratype: *Wiens & Hawksworth 4422*, MO!
= *Phoradendron bolleanum* (Seem.) Eichler.

P. bolleanum, in the limited sense of Trelease (See his Fig. 19a, the fragment) is cited there from Durango as well as Texas and Chihuahua, and is reported from both *Juniperus* sp. and *Arbutus arizonica*. The leaf shape of *P. abietinum* corresponds exactly to that of the Seemann type of *P. bolleanum.*

2. ***Phoradendron acuminatum*** Wiens, Aliso 21(1): 37--38. 2002 [2004], ***nom. illeg.,*** *non P. acuminatum* Kuijt (2003).

Wiens & Hawksworth 4396, holotype US!; isotype RSA! Coll. 1969. Notwithstanding the listing in Wiens and Hawksworth, there are no isotypes at MEXU or MO.

The above specimen corresponds to the type of *P. bolleanum* (Seem.) Eichler.

3. ***Phoradendron flavomarginatum*** Wiens, Aliso 21(1): 38. 2002 [2004], ***syn. nov.***
= *Phoradendron bolleanum* (Seem.) Eichler.
Wiens 7779; holotype US!; isotypes MEXU!, MO! Coll. 1995.

4. ***Phoradendron hawksworthii*** Wiens, Aliso 21(1): 38--39. 2002 [2004], ***syn. nov.***
= *Phoradendron* bolleanum (Seem.) Eichler.
Hawksworth, Lightle & Lampi 1044; holotype US!; isotype RSA! Coll. 1967.

5. ***Phoradendron olivae*** Wiens, Aliso 21(1): 39-40. 2002 [2004], ***syn. nov.***
= *Phoradendron minutifolium* Urban.
Wiens, Hawksworth, Cházaro, & Oliva 7051; holotype IBUG; isotype FPF.
Paratypes: *Cházaro et al. 4479*, IBUG; *De la Rosa, Villareal & Tamayo 1677,* IBUG.

The mensural differences beween *P. olivae* and *P. minutifolium* are too slight to warrant specific distinction, and fall comfortably within the description of the latter species in Kuijt (2003). In fact, the type of *P. olivae* was already listed there under *P. minutifolium*, which is also known from Coahuila and Chihuahua.

As discussed in Kuijt (2003: 124 & 303), there are known hybrids between *P. juniperinum* and *P. densum* that are remarkably similar to *P. minutifolium*. It is not impossible that some of the known populations reported for the latter species are of a similar origin.

6. ***Phoradendron rufescens*** Wiens, Aliso 21(1): 40. 2002 [2004], ***syn. nov.***
= *Phoradendron tetracarpum* Kuijt.
Wiens, Hawksworth, Bailey & Mathiasen 5244; holotype US!; isotypes FPF, RSA! MO! Coll. 1975.

Because of the fact that the US sheet designated by Wiens as holotype (US 3685160, image 01268625) bears two separate specimens, one a female and the other a male, the present International Code of Botanical Nomenclature requires the selection of a lectotype (Art. 9.9, Note 3). (Dr. K. Gandhi (GH) considers that the combination should be regarded as the validly published holotype, and reports that a proposal to cover such cases will be made for the next Code. However, because of the uncertainty of its acceptance, I consider the (herewith) designation of the male (upper right) element as lectotype justified provisionally.)

It is fortunate that the US material of *P. rufescens* includes both a fruiting and a male specimen, allowing for the recording of some missing information for *P. tetracarpum*. The species is profusely branched. Male inflorescences consist of two fertile internodes each; a fertile bract on the proximal fertile internode subtends as many as 10 flowers, and about 5 on the much smaller distal internode. Flowers are regularly triseriate and orange-brown in color. Fruits are 4--5 mm in diameter and reddish-pink. The species appears to be limited to the southern portion of the Sierra Madre Occidental; collections are now known from Hidalgo, Querétaro, and San Luis Potosí.

7. ***Phoradendron sedifolium*** Wiens, Aliso 21(1): 40-41. 2002 [2004], ***syn. nov.***
= *Phoradendron bolleanum* (Seem.) Eichler.
Wiens, Hawksworth, Player & Hermann 5012; holotype US!; isotype RSA!
Notwithstanding the statement in the protologue, no isotype exists at MO. Coll. 1975.

8. ***Phoradendron calvinii*** Wiens, Aliso 21(1): 41. 2002 [2004]. – Type: Mexico. Jalisco, Mpio. Minatitlán, Cerro Grande, 18 km N of Colima Hwy. on Rd to Terrero, elevation 2060 m, *Wiens 7774* in 1995. The holotype is stated to be at US, with isotypes at IBUG, MEXU, UC, FPF, MO, RSA.

The holotype at US as well as the isotypes at MEXU, MO, and UC are missing, and I have not been able to resolve the issue at IBUG and FPF. A neotype therefore needs to be designated. Since Wiens, in 2012, (erroneously) annotated all four of these sheets as "type specimens", it is appropriate to follow his intent, and *Wiens 7781* is herewith designated as neotype:

Mexico. Jalisco. Minatitlán. Sierra de Mammitlán, Cerro Grande, Rd. from El Sauz to Terrero, 1 km SW of Terrero. 1950 m, 6196 ft. 1 Oct 1995, *D. Wiens & C. L. Calvin 7781*. Neotypes at MEXU!, MO!, UC!, US! and presumably at FPF and RSA. "Obligate epiparastite on several other species of *Phoradendron*."

Wiens & Calvin 7781 is not listed in the protologue of *P. calvinii* but, as stated above, was erroneously annotated as the *P. calvinii* "type" by Wiens, as annotated by John Boggan at US. *P. calvinii* is said to be similar to "*P. calyculatum*", a superfluous name for what is now known as *P. falcatum* Eichler; see the discussion in Kuijt (2003: 193).

It should be noted that *Wiens 7774* and *Wiens & Calvin 7781* have different collection data, showing that the confusion is not due to a simple numerical error.

Amended description -- Large, leggy plants said to form pendulous masses to 2 m long, short-bristly on all parts, especially on major leaf veins. Internodes to 11 cm long, terete when fresh, finely grooved when dry, somewhat keeled and expanded distally; basal cataphylls absent; lowest foliar organs on laterals in median position; prophylls inconspicuous. Leaf blade narrowly ovate, tapering distally to a narrowly rounded apex, to 14 cm long and 5 cm wide, base contracted into 1–-1.5 cm long petiole. Venation palmate, conspicuous, with 5 major veins, the outer pair reaching into the mid-leaf area, the central three reaching the apex or nearly so. Dioecious, the neotype female; male plant not seen. Inflorescences axillary; peduncles of female inflorescence ca. 10 mm long, terete, followed by 2 or 3 fertile internodes, the lowest one ca. 1.5 cm long, the distal one less than half as long; flowers ca. 45 per (main) fertile internode, which is densely golden-bristly on all parts, completely ensheathed by the crowded flowers, these without evident seriation. Back of petals with long bristles, but ovary essentially glabrous.

The male inflorescence is stated by Wiens to be narrow, reaching 9--11 cm in length, with 3--5 fertile internodes. Fruits are said to be 3 mm in size, white. The host was stated to be *Phoradendron longifolium* Eichler ex Trel., which ranges from Oaxaca to Sonora (Kuijt 203). However, the (female) neotypes seen have inflorescences no more than 2 cm long, consisting of only 2 or 3 fertile internodes. It is entirely possible, as is the case with some other species, that the mature male inflorescence is much longer, in accordance with Wiens' description.

P. calvinii appears to be a close relative of the widespread *P. falcatum* Eichler, resembling it in its epiparasitism, lack of basal cataphylls, and in the crowded condition of the numerous flowers on fertile internodes. The two species differ in leaf morphology and venation, length of inflorescences and, most strikingly, in the indumentum of especially the flowers in *P. calvinii*, *P. falcatum* being glabrous.

9. ***Phoradenron chazaroi*** Wiens, Aliso 21(1): 41--42. 2002 [2004], ***syn. nov.***
= *Phoradendron dipterum* Eichler.
Wiens, Cházaro, Hawksworth & Oliva 7047; holotype US!; isotypes MEXU!, MO!, RSA! Coll. 30 July 1989.

10. ***Phoradendron durangense*** Wiens, Aliso 21(1): 42. 2002 [2004], ***syn. nov.***
= *Phoradendron falcatum* Eichler.
Wiens & Calvin 5993; holotype US!; isotypes MO!, RSA! Coll. 1985.

P. durangense appears to be but a stout *P. falcatum* as defined in my monograph. The former's protologue is in substantial agreement with my description and illustration (Kuijt 2003, Fig. 100) even though the leaves of *Wiens & Calvin 5993* are somewhat wider. The authors also refer to *P. durangense* as having large, pendulous masses 2 m or more in length, and being parasitic on *P. longifolium* Eichler ex Trel., again in agreement with *P. falcatum*. *P. falcatum* has not previously been reported for southern Durango but its presence there is scarcely a surprise.

11. ***Phoradendron galeanum*** Kuijt, Syst. Bot. Monogr. 66: 212--213, 2003, ***syn. nov.***

This species was described on the basis of a single, slender-leaved specimen collected in Nuevo León. It was subsequently realized that it corresponds to *P. lanceolatum* Engelm., which ranges from Oaxaca to Nuevo León, its type also being derived from the latter state, and it is herewith regarded as a synonym of *P. lanceolatum*. The species is one of the most northerly cataphyllous members of the genus except for *P. californicum*, in which the occurrence of cataphylls is irregular (Kuijt 1997).

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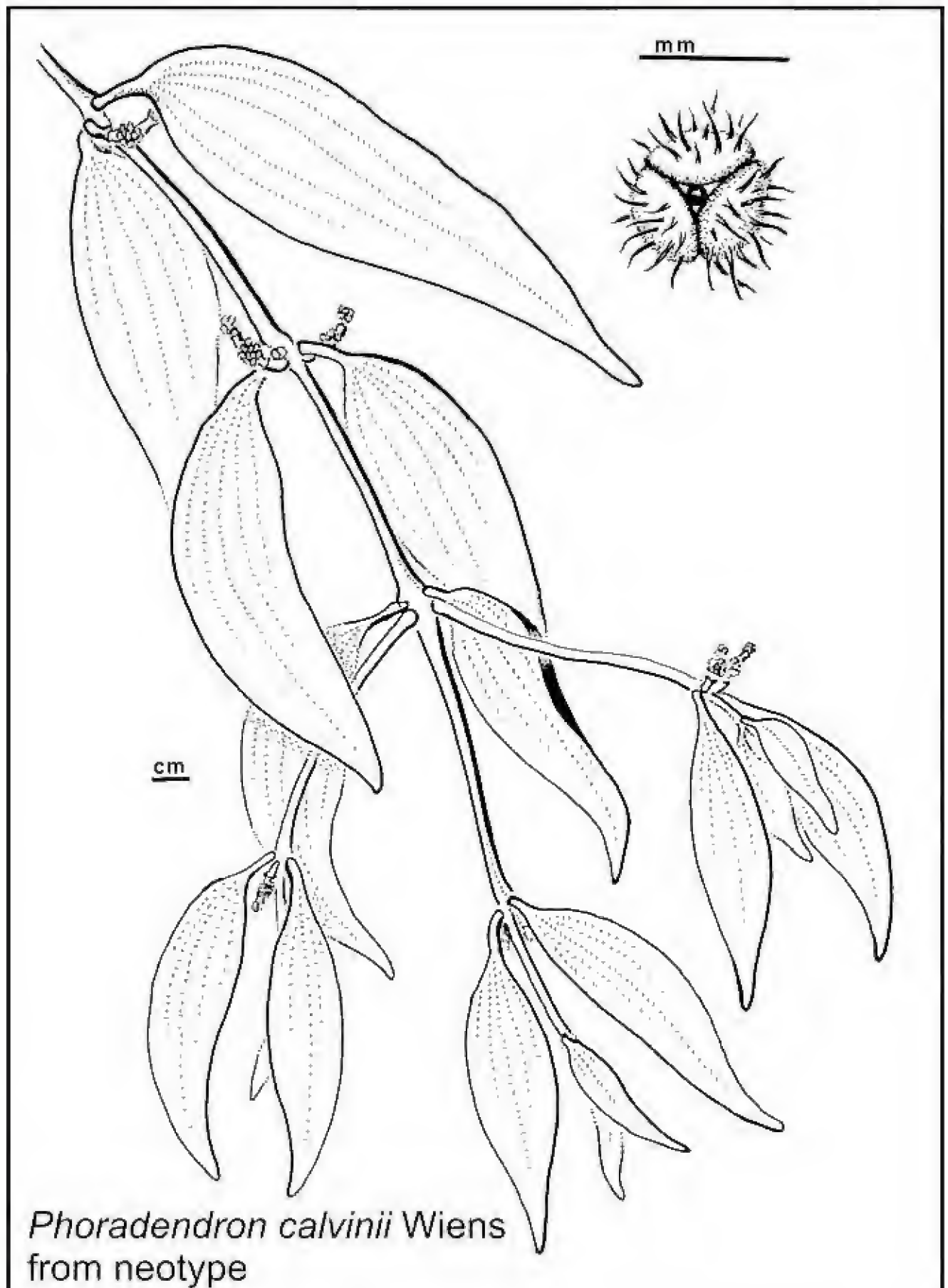

Fig. 1. *Phoradendron calvinii* Wiens (UC neotype). Habit and enlarged flower.

Comparison of a Managed and Unmanaged *Quercus stellata* (post oak) Community

J. K. Bush
College of Sciences, University of Texas at San Antonio
One UTSA Circle, San Antonio, TX 78249, USA
janis.bush@utsa.edu

and

O. W. Van Auken
Department of Biology, University of Texas at San Antonio
One UTSA Circle, San Antonio, TX 78249, USA
oscar.vanauken@utsa.edu

ABSTRACT

The woody vegetation of two equal aged, adjacent, *Quercus stellata* (post oak) woodlands was examined. One community was managed for the past 30 years by manual removal of encroached *Juniperus ashei* (Ashe juniper=mountain cedar) plants and hunting *Odocoileus virginianus* (white-tailed deer) and the second community was unmanaged. The communities were simple with only three tree size species found in both. In the managed community, *Q. stellata* had the highest tree density (210 plants/ha), basal area (16.67 m^2/ha) and importance (98%) with *Celtis laevigata* (sugar hackberry) and *Ulmus crassifolia* (cedar elm) being minor species. In the unmanaged community, *J. ashei* had the highest tree density (1,856 plants/ha), basal area (16.16 m^2/ha) and importance (74%) with *Q. stellata* second in tree density (144 plants/ha), basal area (12.67 m^2/ha) and importance (25%) and *Bumelia lanuginosa* (gum bumelia) a minor species. Total tree density was almost ten times higher in the unmanaged community and basal area was almost twice as high, because of the presence of *J. ashei* density (1,856 plants/ha) and basal area (16.16 m^2/ha). Total juvenile density was more than five times higher (64,549 plants/ha) in the managed community with *U. crassifolia*, *Smilax bona-nox* (bull briar, a woody vine) and *C. laevigata* being the woody species with the highest density. In the unmanaged community *B. lanuginosa*, *U. crassifolia* and *S. bona-nox* had the highest densities. *Quercus stellata* trees had a unimodal distribution in both communities, but no evidence of recruitment of juveniles into the adult population. The Weibull shape statistic (c) suggested normal distributions in both communities. *Juniperus ashei* had a unimodal distribution, a negative exponential function in the unmanaged community. There were six juvenile tree species including *Q. stellata* in the managed community and five in the unmanaged community, but only *J. ashei* was being recruited into the adult population and only in the unmanaged community. Based on size distributions, *Q. stellata* and *J. ashei* do not seem to be ecologically compatible. Although the *O. virginianus* population may be influencing recruitment of *Q. stellata*, another factor besides the presence of *J. ashei*, such as the biomass or density of the understory C_4 grasses, may be interfering with recruitment of *Q. stellata* into the adult *Q. stellata* population. Published on-line www.phytologia.org *Phytologia 97(4): 252-264 (Oct 1, 2015)*. ISSN 030319430.

KEY WORDS: community composition, density, basal area, community manipulation, diameter distributions, Weibull distributions, recruitment failure.

Community structure and species occurrence in a particular area are determined by the tolerance or requirements of the species to abiotic and/or biotic conditions present. However, the tolerance to or requirements for these conditions are at least slightly different for each species. Categorizing the characteristics or factors that determine why one species is present or dominant where it is found and not in another place or habitat is much more challenging (Begon et al. 2006). Several different conclusions

can be drawn when two woody species are present in the same place with one dominating the overstory and the other dominating the understory. One conclusion would be that the understory species is encroaching into the overstory species' space with the ultimate loss of the overstory species. A second conclusion could be that the overstory species encroached on the understory species, had a faster growth rate, and will result in the ultimate loss of the understory species. A third conclusion could be that they both arrived at the same time and they coexist with few adverse interactions. However, with long lived woody species the correct conclusion is difficult to clarify. The sequence of woody species is hard to determine but is occurring during community succession (Begon et al. 2006).

Central Texas plant communities, including *Quercus stellata* (post oak) communities, have no doubt been subject to current warming trends, plant community migrations, and land management practices. Land management means different things to different people including managers. One can manage for or against various species of plants and/or animals (Scifres 1980). These practices will lead to communities with a different plant and animal composition in the future.

Historically we know that plant communities worldwide have changed many times in the past (see Berner 2005). Environmental conditions appear cyclic and they have been connected to global scale fluctuations in the climate of the Earth over the past 420,000 years. Evidence of these changes have been found in ice cores and other temperature proxies taken from thick glacial ice sheets (Imbrie and Imbrie 1980). At least four cycles of warming and cooling have been found in the ice cores (Petit et al. 1999). In addition, over the last 11,000-15,000 years, the end of the last glacial maximum or cold cycle, the migration of North American plant communities has been linked to the retreat of the Laurentide glaciers (Delcourt et al. 1983; Betancourt et al. 1990; Van Devender 1995).

It is well documented that plant communities have changed; these changes also appear to be related to global climate. In addition, plant communities will continue to change in the future as they respond to changes in environmental conditions (McDowell et al. 1995; Berger and Loutre 2002; Ehleringer 2005). On a smaller scale, land management strategies can affect changes in plant communities and further complicate the picture. In most ecological studies the land management history of a particular community is not precisely known (Van Auken 2009).

Climate and plant communities are not constant and no doubt they will continue to be modified in the future. For example, plant communities are modified as woody plants encroach into grasslands, grass biomass, density and cover decline as woody plant biomass, density, and cover increase (see Van Auken 2009). In addition, there is a shift in location of the biomass, species richness, and diversity as herbaceous species are replaced by woody species. The processes involved are dynamic and the direction of the changes can be reversed. In addition to the changes in grassland communities, woodland communities change as well. Succession in central Texas woodland community has been studied (Van Auken and Bush 2013). Early woodland succession typically goes from various shade intolerant woody legumes to various shade tolerant trees. However, in the current study, a shade intolerant species (*Juniperus ashei*) (McKinley and Van Auken 2005; Van Auken et al. 2005; Grunstra 2008) is present below the canopy of a potentially shade tolerant species (*Q. stellata*). This seems to be the reverse of typical succession patterns driven by light availability (Begon et al. 2006).

Most plant community modifications in the distant past were relatively gradual compared to changes seen in the past 200-400 years. The causes of the recent modifications in plant communities could be diverse, but seem to be the result of high levels of domestic herbivory coupled to reduced fire intensity and frequency (see Van Auken 2009). We had an opportunity to examine two adjacent, equal-aged *Quercus stellata* communities on the western edge of its range with very different management histories (Figure 1). One community was unmanaged while the other was managed. Management consisted of continuous removal of all *J. ashei* plants and a reduction of large herbivores by hunting.

PURPOSE

The purpose of the present study was to ecologically describe a managed *Quercus stellata* (post oak) woodland community on the Cibolo Preserve in Boerne, Texas and to compare this community to an adjacent, unmanaged *Q. stellata*/*J. ashei* (post oak/mountain cedar-Ashe juniper) woodland. We hypothesize that if competition from *J. ashei* and herbivory from *O. virginianus* were affecting the recruitment of *Q. stellata* into the adult population, that we would see differences in the *Q. stellata* size class distribution.

SPECIES

Quercus stellata is widespread in central and east Texas, and across the eastern U. S. (Figure 1). In Texas it is found in a band of sandy soil called the Post Oak Savanna that extends from the Rio Grande through east central Texas north into Oklahoma (Correll and Johnston 1979). In addition, it is found in the Cross-timbers region of north Texas along with other *Quercus* species. In the Edwards Plateau Physiographic Region it is found in scattered populations usually mixed with *J. ashei* that is present throughout central Texas (Van Auken 2016). The northeastern Edwards Plateau Region, an area called the Lampasas Cut-Plane, is a partially forested area, where *Q. stellata* and various other *Quercus* species are found in woodland and forested areas (Gehlbach and Amos 1988). A study of weather patterns in southern Oklahoma, north, central and south Texas used old *Q. stellata* trees to develop a climate sensitive tree ring chronology (Stahle and Cleaveland 1988).

METHODS

Characteristics of the woody vegetation within a managed 12.1 ha *Q. stellata* woodland and an unmanaged, equal aged 4.5 ha *Q. stellata*/*J. ashei* woodland were determined using the quadrat procedure (Chambers and Brown 1983; Bonham 1989; Van Auken et al. 2005). The communities were adjacent but separated by a deer-proof fence. The understory of the managed community had a high cover of warm-season or C_4 grasses while the unmanaged community had little grass cover except in intermittent canopy openings of gaps. One community was on the 250 hectare Cibolo Preserve in Kendall County, central Texas (29.7691°N and 98.6935°W, Figure 1) and one was adjacent to it, but not on the preserve. Measurements for trees were done in 5 m by 5 m or 25 m^2 quadrats established along a belt transect line and all trees of all species were identified, counted, and measured. A tree was considered any woody plant greater than 1 cm in diameter at 1.54 m (breast height) except for *J. ashei*. *Juniperus ashei* had multiple stems and was measured at the base, just above ground level. If *J. ashei* plants were greater than 1.54 m tall, with a basal diameter greater than 1 cm diameter they were considered trees; otherwise, they were considered juveniles. Seedlings (< 0.5 m in height) and saplings (> 0.5 m in height, and < 1 cm in diameter) were juveniles, and identified and counted in 5 randomly placed 1 m^2 quadrats within each 25 m^2 quadrat.

Trees were sampled in a total of 204-25 m^2 quadrats in the managed community and 50-25 m^2 quadrats sampled in the non-managed community. In the managed community, juvenile woody plants were sampled in 1,020-1 m^2 quadrats and 250-1 m^2 quadrats in the unmanaged community. For trees, density stabilization curves indicated sample adequacy (Figures 2 and 3) (see Van Auken et al. 2005). Number of individual trees of each species in each 25 m^2 quadrat was counted and recorded and the diameter of each adult (tree) was measured with a tree caliper. The number of juveniles of each woody species in each of five 1 m^2 randomly distributed sub-quadrats within the larger quadrat was counted and recorded. Density of seedlings and saplings per species was calculated and pooled as juveniles. Total tree and juvenile density was calculated separately for each community by summing the density of each species. Total tree basal area was calculated by summing the basal area for each species. Density and

basal area were used to describe the plant communities as was importance (% density + % basal area/2). In addition, the density of all species of juveniles was calculated for the same purpose.

Some community characteristics were compared with a X^2-test (Sall et al. 2012). From the tree data, diameter size class histograms were constructed. This is a quantitative way to examine population size structures using a two-parameter Weibull function fitting the diameter distributions using a maximum-likelihood algorithm (Cohen 1965; Bailey and Dell 1973; Ryniker et al. 2006; Van Auken et al. 2007). These distributions can take various shapes determined by c. If c is < 1, the plot is steeply descending and monotonic. If c =1, then the plot is a negative exponential distribution. When c is > 1, the function is unimodal. When 1 < c < 3.6 the distribution has a skew that is positive. If c = 3.6 the distribution is roughly normal. A negatively skewed distribution is indicated if c > 3.6. The scale (b) is called the "characteristic life" and is related to the mean of the distribution (Knox et al. 1989).

RESULTS

Based on the number of tree species found (Table 1), the two communities were simple, with three species of trees found in both. In the managed community, *Q. stellata* had the highest tree density (210 plants/ha), basal area (16.67 m^2/ha) and importance (98%) with *Celtis laevigata* (sugar hackberry) and *Ulmus crassifolia* (cedar elm) being minor species. In the unmanaged community, *J. ashei* had the highest tree density (1,856 plants/ha), basal area (16.16 m^2/ha) and importance (74%); *Q. stellata* had a tree density of 144 plants/ha, basal area of 12.67 m^2/ha, and importance of 25%; and *Bumelia lanuginosa* (gum bumelia) was a minor species. Total tree density was significantly different ($X^2 < 0.05$) and 9.42 times higher in the unmanaged community. Total basal area was significantly different ($X^2 < 0.05$) and 1.7 times higher, because of the presence of *J. ashei*. *Quercus stellata* density in the managed community was significantly different ($X^2 < 0.05$) and 210 plants/ha compared to the unmanaged community that was 144 plants/ha, a 31% reduction. *Quercus stellata* basal area was reduced by 24% in the unmanaged community.

The total juvenile density in the managed community was significantly different ($X^2 < 0.05$) and 5.34 times higher (64,549 plants/ha) compared to the unmanaged community (12,080 plants/ha) (Table 2). In the managed community in descending order, the species with the highest juvenile densities were *U. crassifolia*, *Smilax bona-nox* (bull briar, a woody vine) and *C. laevigata*. In the unmanaged community in descending order, *B. lanuginosa*, *U. crassifolia* and *S. bona-nox* had the highest densities. *Quercus stellata* juvenile density in the managed community was significantly different ($X^2 < 0.05$) at 1,833 plants/ha, compared to the unmanaged community and fourth highest in this community at 6% relative density. In the unmanaged community it had the lowest juvenile density of the six woody species found at 120 plants/ha or a relative density of 1%.

Quercus stellata trees had a unimodal distribution in both communities (Figure 4 A and B), but no evidence of recruitment into the adult population. The Weibull shape statistic (c) suggested normal distributions in both communities, with a slightly negative skew (Figure 4A) and a slightly positive skew (Figure 4B) and very few small diameter individuals. Means were similar but 3% smaller in the unmanaged community. *Juniperus ashei* had a unimodal distribution in the unmanaged community (Figure 4 C), but the distribution was a negative exponential function with a positive skew. There were 85 *J. ashei* individuals in the two smallest diameter size classes and no *Q. stellata* plants in these size classes. There were a number of juveniles of both *Q. stellata* and *J. ashei* (Table 2) but only *J. ashei* juveniles were being recruited into the adult population and only in the unmanaged community. There were six juvenile tree species in the managed community and five in the unmanaged community, but only *J. ashei* was being recruited into the adult population and only in the unmanaged community.

DISCUSSION

No one had examined the replacement dynamics of *Quercus stellata* until this current study. The central Texas *Q. stellata* populations that we examined do not appear to be replacing themselves in either community examined (Figure 4). This is not unusual because there are a number of *Quercus* species, with many populations that are not replacing themselves (McCune and Cottam 1985; Pallardy et al. 1988; Reich et al. 1990; Beck 1992; Abrams 2003). These population changes appear to be geographically widespread (Lorimer 1992) and species independent (Loftis and McGee 1992). Many *Quercus* species have been significant components of North American forests for the last 10,000 years (Craig 1969; Watts 1979; Delcourt and Delcourt 1985; Abrams 1992; Abrams 2003). However, population changes today are associated with a lack of recruitment or replacement of the mature *Quercus* trees (Shumway et al. 2001; Cowell and Hayes 2007). Size-distributions and comparative studies have indicated recruitment failure in *Q. alba* (white oak), *Q. bucklei* (Texas red oak), *Q. gambelii* (Gambel's oak), *Q. lobata* (Garry oak), and *Q. rubra* (red oak) (Russell and Fowler 2002; Aldrich et al. 2005; Ryniker et al. 2006; MacDougall 2008). While this recruitment failure is well documented, the causes may be species or location dependent (Lorimer 1992).

Recruitment failure - the failure of juvenile plants to reach maturity - has been described for many *Quercus* species and is a major concern for conservation biologists and land managers. Recruitment failure can be due to many factors usually delineated as either pre-germination or post-germination. Previous studies have reported high *Quercus* juvenile densities, indicating that seed production and seed germination, or pre-germination factors, are not the reasons for recruitment failure (see Ryniker et al. 2006). Therefore, post-germination influences are likely the cause of recruitment failure.

Light, moisture, temperature, and fire are post-germination, abiotic factors which have been shown to influence *Quercus* establishment and growth (Nathan and Muller-Landau 2000; Collins 2003; Haas and Heske 2005; Van Auken and Bush 2009). Biotic factors, such as competition from other plants and herbivory by various animal populations have also been shown to affect establishment and growth of various *Quercus* species. Juveniles are frequently numerous under mature trees, however often these juveniles are not competitive with juveniles of other plant species (Lorimer et al. 1994). It is widely accepted that herbivores affect regeneration through a number of mechanism, including direct browsing or indirectly by browsing associated species (Shumway et al. 2001; Cowell and Hayes 2007). *Odocoileus virginianus* (white-tailed deer) populations in central Texas have increased dramatically (Doughty 1983) and at approximately one deer/5 ha populations are the highest in the United States (Armstrong and Young 2000; Fulbright and Ortega-S. 2006). However, in the managed community, *O. virginianus* populations were reduced to approximately one deer/7.2 ha. This population which is 31% lower than native communities should have a reduced effect on the population of various deciduous species, including *Q. stellata*. However, we did not find *Q. stellata* recruitment in either community.

This high *O. virginianus* population is probably because of major carnivore reductions (Beschta and Ripple 2009; Dirzo et al. 2014). Herbivory has been shown to produce a pronounced reduction in juvenile deciduous species survival and decreased sapling density in diverse North American *Quercus* savannah and woodland communities (McCune and Cottam 1985; Pallardy et al. 1988; Reich et al. 1990; Beck 1992; Abrams 2003). In spite of this recruitment failure, productivity and biomass of these communities is probably determined by the large old trees that will be present in these communities for many years (Eisen and Plotkin 2015).

We anticipated a lack of *Q. stellata* recruitment in the unmanaged *Q. stellata*/*J. ashei* woodland community because of potential interaction of the two species (Figure 4 B and C) and the presence of large herbivores. However, we did not anticipate a lack of recruitment in the managed community where *J. ashei* plants were removed and many of the large herbivores were removed as well. *Odocileus*

virginianus populations of one deer per 3.2 to 5.0 hectares will reduce *Quercus* seedlings (personal observation J. Jackson and wildlife biologist Kevin Meier). Consequently, the *O. virginianus* population on the preserve may still be high enough to prevent *Q. stellata* juveniles from becoming part of the adult population. This suggests another factor or series of undefined factors are controlling *Q. stellata* recruitment. If the recruitment is intermittent or cyclic, the factor controlling the recruitment will be much harder to identify. If there are multiple factors that have to be present together or in sequence they will also be difficult to identify. The presence of a high density or cover of various warm season or C_4 grasses below the *Q. stellata* canopy may require manipulation to release nutrients required by the juveniles so they can grow into the canopy as replacements. Possibly certain nutrients present in the C_4 grasses are currently unavailable to the juveniles and this could be the unknown key.

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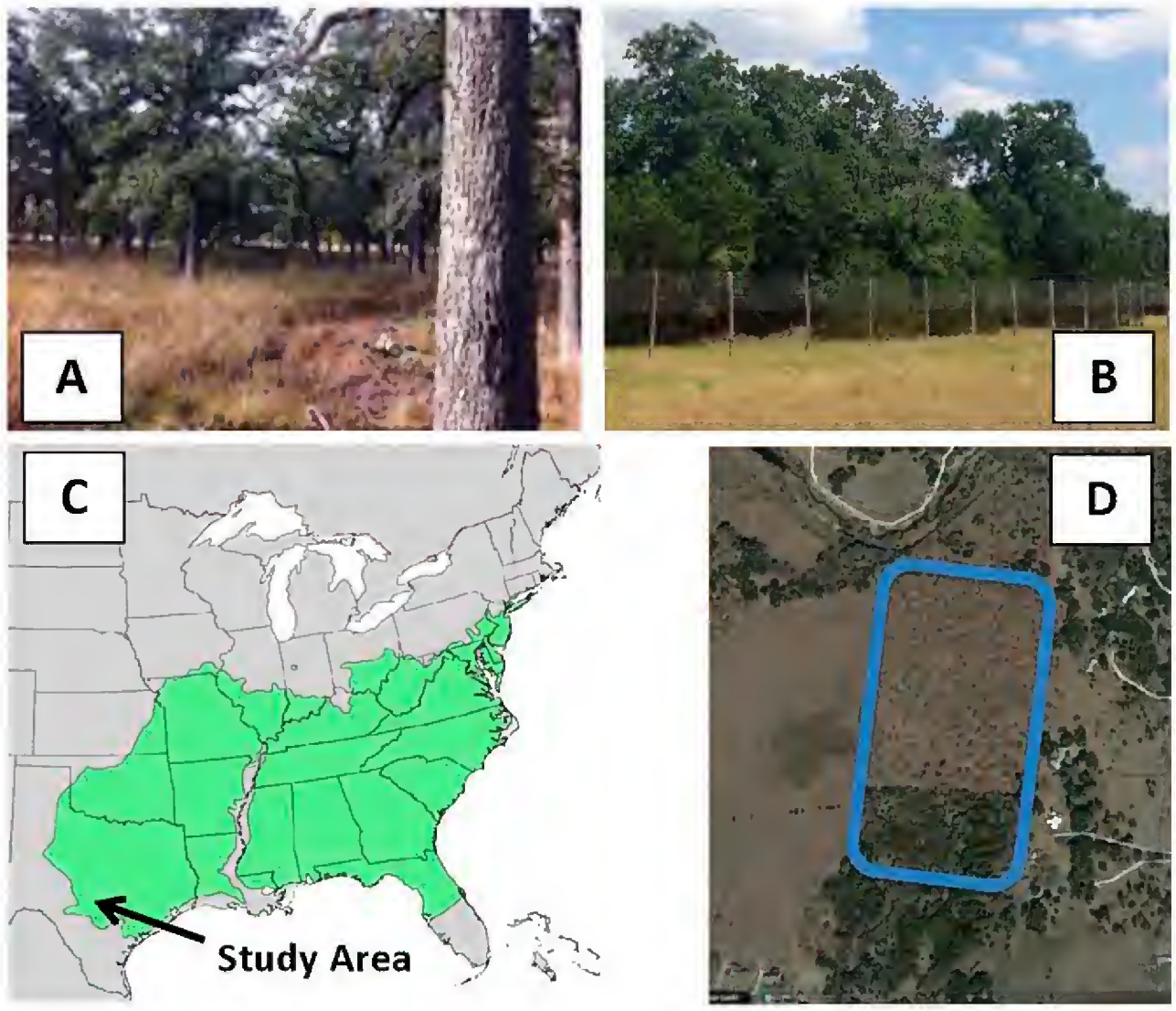

Figure 1: Two *Quercus stellata* savannas in central Texas: A) Managed with the *Juniperus* trees and shrubs removed by hand and many large herbivores removed and B) the edge of a post oak savanna in close proximity to the previous community with no manuplation. C) A map shows the distribution of *Quercus stellata* with the arrow approximately indicating the study area and D) an aerial photograph shows the *Quercus stellata* community outlined. The upper part is the managed community (light, winter, no leaves) and the lower is the non- managed community (dark, juniper folage). Upper photographs were taken by J. K. Bush.

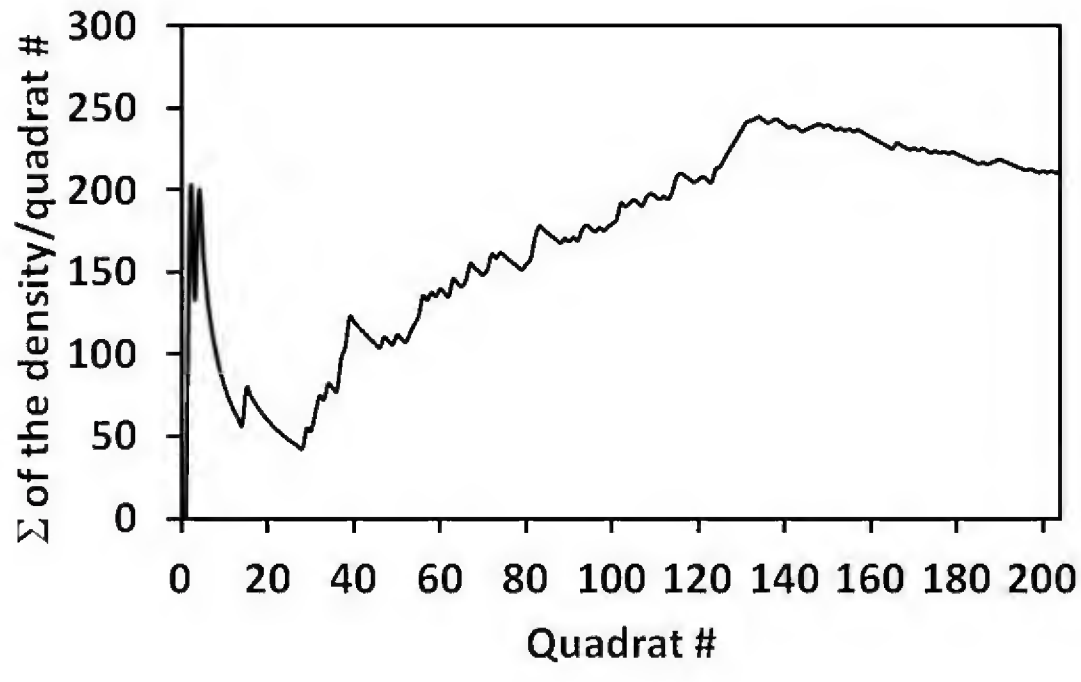

Figure 2: Density stabilization curve is presented for estimating sample adequacy for the *Quercus stellata* heavily managed community at the Cibolo Preserve, Kendall County, Boerne, Texas.

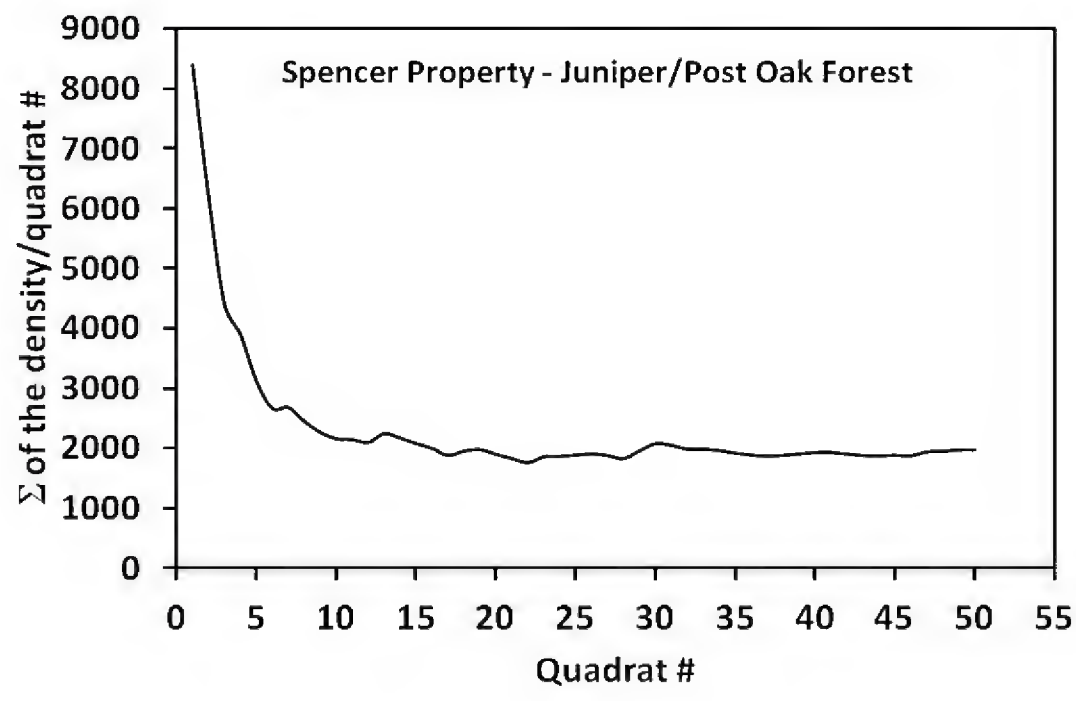

Figure 3: Density stabilization curve is presented for estimating sample adequacy for the *Quercus stellata*/*Juniperus ashei* unmanaged community near the Cibolo Preserve, Kendall County, Boerne, Texas.

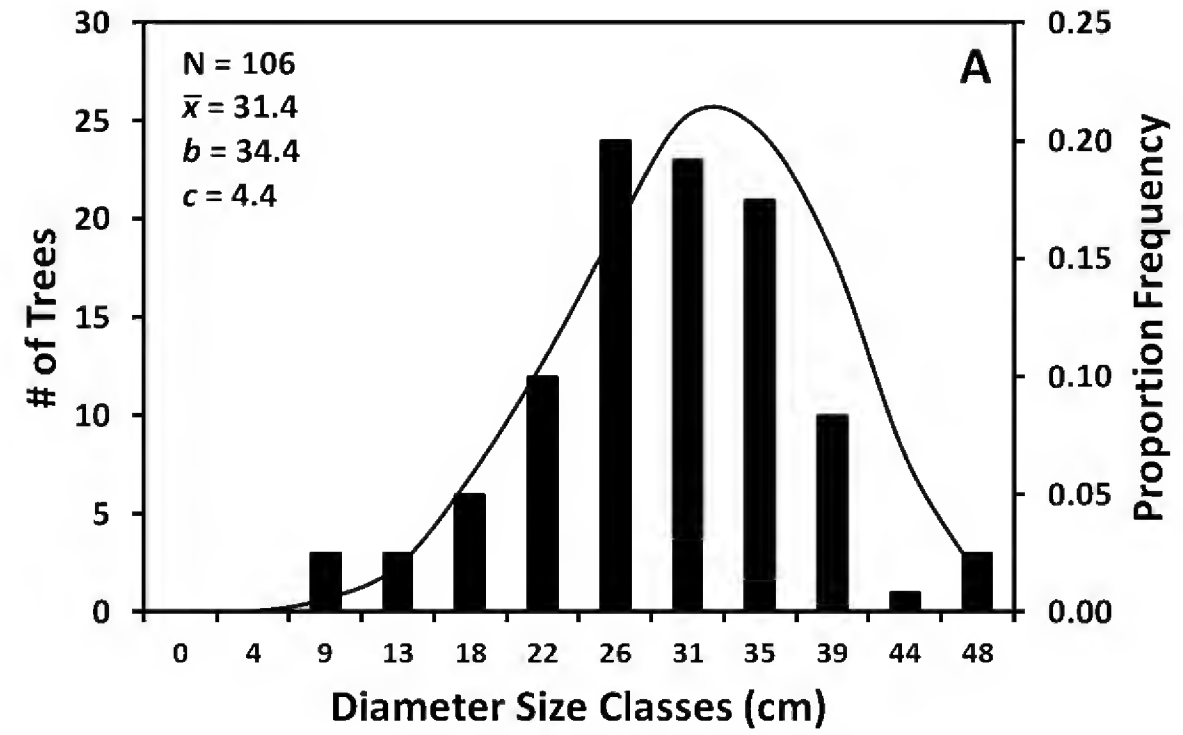

Figure 4: Diameter size class histograms and fitted Weibull distributions (solid lines) are presented. The top histogram (A) is for *Quercus stellata* in the managed community. The middle and lower histograms (B and C) are for *Quercus stellata* (B) and *Juniperus ashei* (C) in the unmanaged community. The x-axis is 3 to 5 cm in diameter depending of the community and species. The zero for *J. ashei* is actually > 1 cm in diameter. The *y*-axis (left) shows the number of trees counted for each size class and on the right is the proportion frequency for each bar. Sample size (N), mean community species density (*x*-bar), Weibull scale statistic (*b*) and the Weibull shape statistic (*c*) are also presented.

Table 1. Phytosociological data for trees found in a managed *Quercus stellata* community on the Cibolo Preserve, Kendall County, Boerne, Texas and unmanaged *Quercus stellata/Juniperus ashei* community adjacent to the Cibolo Preserve. Density = plants/ha, Basal area = m^2/ha, and % Importance = (% density + % Basal Area)/2. An asterisk next to a value indicates a significant difference between the managed and unmanaged communities for that characteristic.

Managed

Species	Density	% Density	Mean Basal Area	Basal Area	% Basal Area	% Importance
Quercus stellata	210	98	795	16.67	98	98
Celtis laevigata	2	1	1,052	0.21	1	1
Ulmus crassifolia	2	1	749	0.15	1	1
Total	**214**	**100**		**17.03**	**100**	**100**

Unmanaged

Species	Density	% Density	Mean Basal Area	Basal Area	% Basal Area	% Importance
Juniperus ashei	1,856	92	87	16.16	56	74
Quercus stellata	144*	7	880	12.67	44	25
Bumelia lanuginosa	16	1	114	0.18	1	1
Total	**2,016***	**100**		**29.02***	**100**	**100**

Table 2. Phytosociological data of juvenile woody species in a managed Quercus *stellata/Juniperus ashei* woodland community in the Cibolo Preserve, Kendall County, Boerne, Texas and an unmanaged *Quercus stellata/Juniperus ashei* woodland community adjacent to the Cibolo Preserve.

Managed		
Species	**Juvenile Density/ha**	**% Density**
Ulmus crassifolia	21,000	65
Smilax bona-nox	6,389	20
Celtis laevigata	2,389	7
Quercus stellata	1,833	6
Juniperus ashei	222	1
Diospyros texana	194	1
Ilex decidua	111	0
Bumelia lanuginosa	56	0
Berberis trifoliata	56	0
Parthenocissus quinquefolia	28	0
Total	**64,549**	**100**

Unmanaged		
Species	**Juvenile Density/ha**	**% Density**
Bumelia lanuginosa	5,440	45
Ulmus crassifolia	4,280*	35
Smilax bona-nox	1,880*	16
Juniperus ashei	200	2
Celtis laevigata	160*	1
Quercus stellata	120*	1
Total	**12,080***	**100**

Geographic variation in the volatile leaf oil of *Juniperus occidentalis*. II. Analysis from throughout its geographic range

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA email
Robert_Adams@baylor.edu

and

Mark Corbet
7376 Southwest McVey Ave., Richmond, OR 97756

ABSTRACT

Volatile leaf oils of *J. occidentalis* were analyzed from throughout its range. The major differentiation found was the divergence of the Trinity Alps, California population from other *J. occidentalis* populations in having a high amount of sabinene (20.4%) and only a trace of bornyl acetate. Minor variations in the oil compositions were found, these being chiefly on the margins of the range of the species, except for the Burns, Oregon population. The oil of *J. occidentalis* f. *corbetii*, a shrubby form that occurs about 30 km east of Bend, Oregon , was somewhat distinct in having large amounts of p-cymene (20.0%) and bornyl acetate (24.5%). Published on-line www.phytologia.org *Phytologia 97(4): 265-270 (Oct 1, 2015).* ISSN 030319430.

KEY WORDS: *Juniperus occidentalis, Juniperus occidentalis* forma *corbetii,* Cupressaceae, terpenes, geographic variation.

Juniperus occidentalis, *J. grandis* (=*J. occidentalis* var. *australis*) and *J. osteosperma* are three very closely related junipers in the western United States (Vasek 1966; Adams 2011). Adams and Kauffmann (2010a) and Adams (2012a) reported on the geographic variation in the leaf oils and DNA of *J. grandis, J. occidentalis and J. osteosperma.* Recently, Adams (2012b) examined the leaf oils of *J. occidentalis* f. *corbetii* from east of Bend OR and presented a small study of geographic variation in the leaf oils of *J. occidentalis*. Hybridizations between *J. grandis, J. occidentalis* and *J. osteosperma* have been examined by Vasek (1966) and later by Terry et al. (2000) and Terry (2010) using DNA markers and morphology. Analysis of hybridization using leaf terpenes at Leviathan mine, Nevada (Adams 2013a) and Buffalo Hills, northwestern Nevada were recently published by Adams (2013a,b).

Juniperus occidentalis is a narrowly distributed species, growing largely east of the Cascade Mtns. and thence into nw California, eastern Idaho and northwestern Nevada (Fig. 1).

The purpose this paper is to report on a comprehensive analysis of geographic variation in the leaf essential oils of *J. occidentalis*. The reader is referred to Adams (2013a, b) for a summary of the hybridization between *J. occidentalis* and *J. osteosperma.*

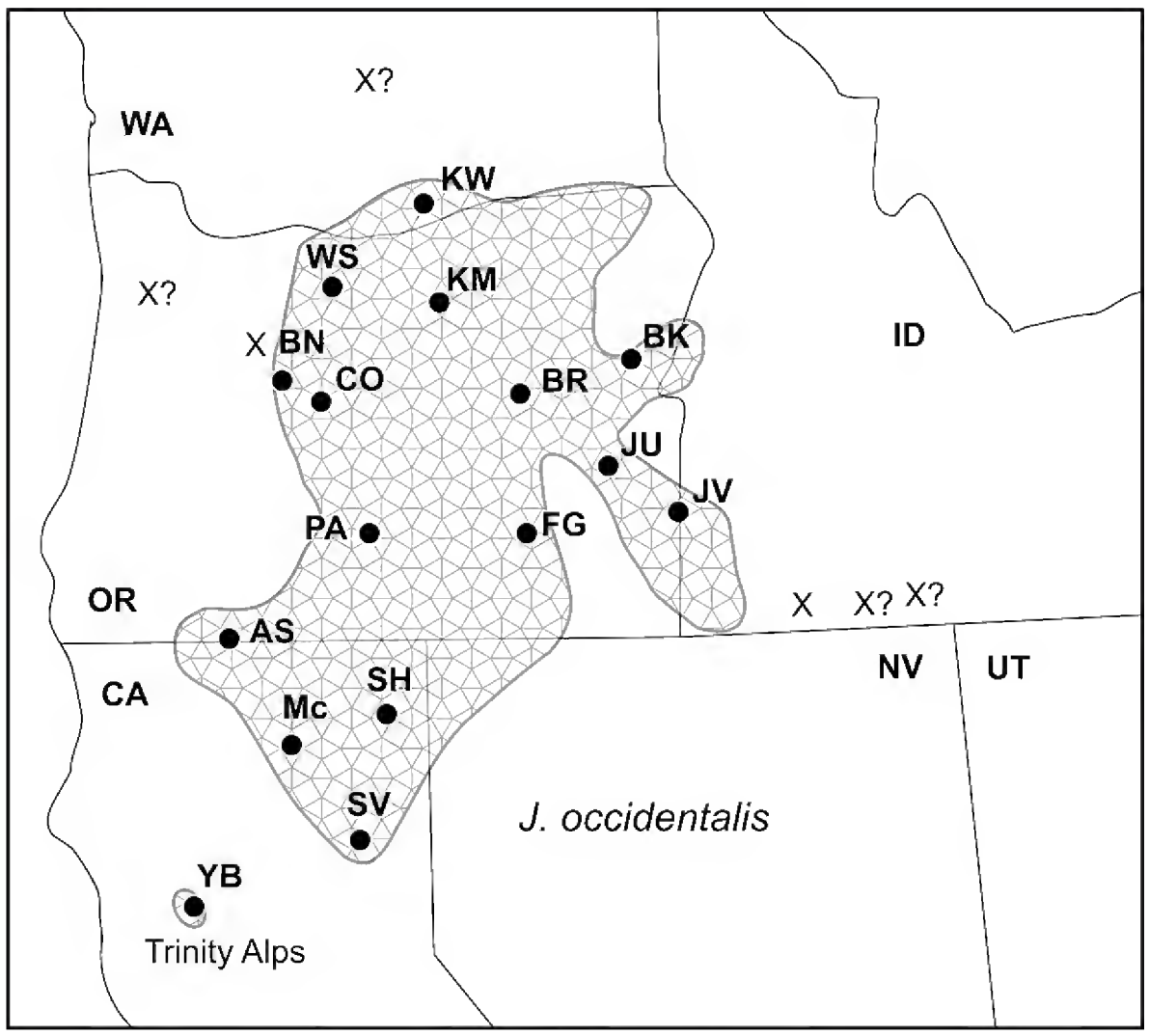

Figure 1. Distribution of *J. occidentalis* modified from Vasek (1966) and Adams (2011). Populations sampled in this study are denoted by shaded circles. Note that the southwestern-most population is at Trinity Alps, CA (Yolla Bolly). X marks disjunct specimens. X? marks questionable reports of *J. occidentalis*.

MATERIALS AND METHODS

Plant material: *J. occidentalis,* **KW** *Adams 11940-11942,* 12 km e of Jct. WA 14 & US 97 on WA 14, 45º 44.392'N, 120º 41.207'W, 170 m, Klickitat Co.; WA, **WS**, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR; 44º 53.676'N, 120º 56.131'W, 951 m, Wasco Co., OR; **BN** *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44º 02.390'N, 121º 20.054'W, 1132 m, Deschutes Co., OR; **AS** *Adams 11952-11954,* 14 km e of Jct. OR66 & I5, on OR 66, 42º 08.044'N, 122º 34.130'W, 701 m, Jackson Co., OR east of Ashland; **Mc** *Adams 11957-11959*, on CA 299, 10 km e of McArthur, CA, 41º 05.313'N, 121º 18.921'W, 1091 m, Lassen Co., CA; **TA** *Adams 11995-11998 (Kauffmann A1-A3, B1),* Trinity Alps, Yolla Bolly-Middle Eel Wilderness, 40º 06' 34"N, 122º 57' 59W, 1815- 2000 m, Trinity Co., CA, **SV** *Adams 12342-12346*, 19 km wsw of Susanville, CA, on CA 36, 40º 22.178'N, 120º 50.211' W, 1570 m, Lassen Co., CA, **SH** *Adams 12347-12351*, on US 395, 5 km n of Madeline near Sage Hen Pass, 41º 05.867'N, 120º 28.456' W, 1695 m, Lassen Co., CA, **JU** *Adams 12242-12244*, 3 mi. w Juntura, OR on OR 20, trees, 3 mi w of Juntura, OR on OR 20, 43° 45' 52.61"N; 118° 08' 40.49"W, 953 m (Corbet 2010-1,2,3). **BR** on US 395/20, 9 mi e of Riley, Harney Co., OR, about 17 mi w of Burns, OR. 43° 31' 56.4" N, 119° 19' 22.3" W, 4555 ft, 24 Aug 2012, OR. *Mark Corbet ns*, *Adams 13512-13516*; **PA** on Co Rd. 2-08, 1.5 mi sw of Paisley, Lake Col, OR. 42° 41' 17.7" N, 120° 34' 10.0" W, 4478 ft, 24 Aug 2012, *Mark Corbet ns Adams 13517-13521*, **FG** 0.3 mi w of Frenchglen, OR on OR 205. 42° 49' 34.7" N, 118° 55' 01.2" W, 5064 ft, 24 Aug 2012, Harney Co., OR, *Mark Corbet ns, Adams 13522-13526,* **JV** 2.8 mi sw of Jordan Valley, Owyhee Co., ID, on Trout Creek Rd., thence 0.5 m ne on dirt road. 42° 57' 50.3" N, 117° 00' 01.5" W, 4477 ft, 24 Aug 2012, *Mark Corbet ns, Adams 13527-13531*, **KM**, scattered, with sage, 1 mi s of Kimberly, Grant Co., OR on OR hwy 19. 44° 44' 58.2" N, 119° 38' 10.5" W, 1909 ft. 31 Aug 2012, *Mark Corbet ns, Adams 13537-13541*, **BK** scattered, with sage, 15.5 mi se of Baker, Baker Co., OR on I 84. 44° 38' 59.4" N, 117° 33' 48.2" W, 3350 ft, 31 Aug 2012, *Mark Corbet ns Adams 13542-13546.*

J. occidentalis f. *corbetii* R. P. Adams. **CO** *Adams 11949-11951,* 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43º 53.922'N, 120º 59.187'W, 1274 m, Deschutes Co., OR. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The volatile leaf oil of *J. occidentalis* is dominated by sabinene, p-cymene and bornyl acetate (Table 1). The leaf oil from the Trinity Alps (Yolla Bolly) population is atypical in having more sabinene (20.4%) and only a trace of bornyl acetate. The *J. o.* f. *corbetii* shrubs east of Bend, OR have large amounts of p-cymene (20.0) and bornyl acetate (24.5%).

To visualize the overall similarities in the leaf oils, a minimum spanning network was constructed (Figure 2). The major differentiation is the divergence of the Trinity Alps (Yolla Bolly) population (Fig. 2). Minor variation is seen among the other populations.

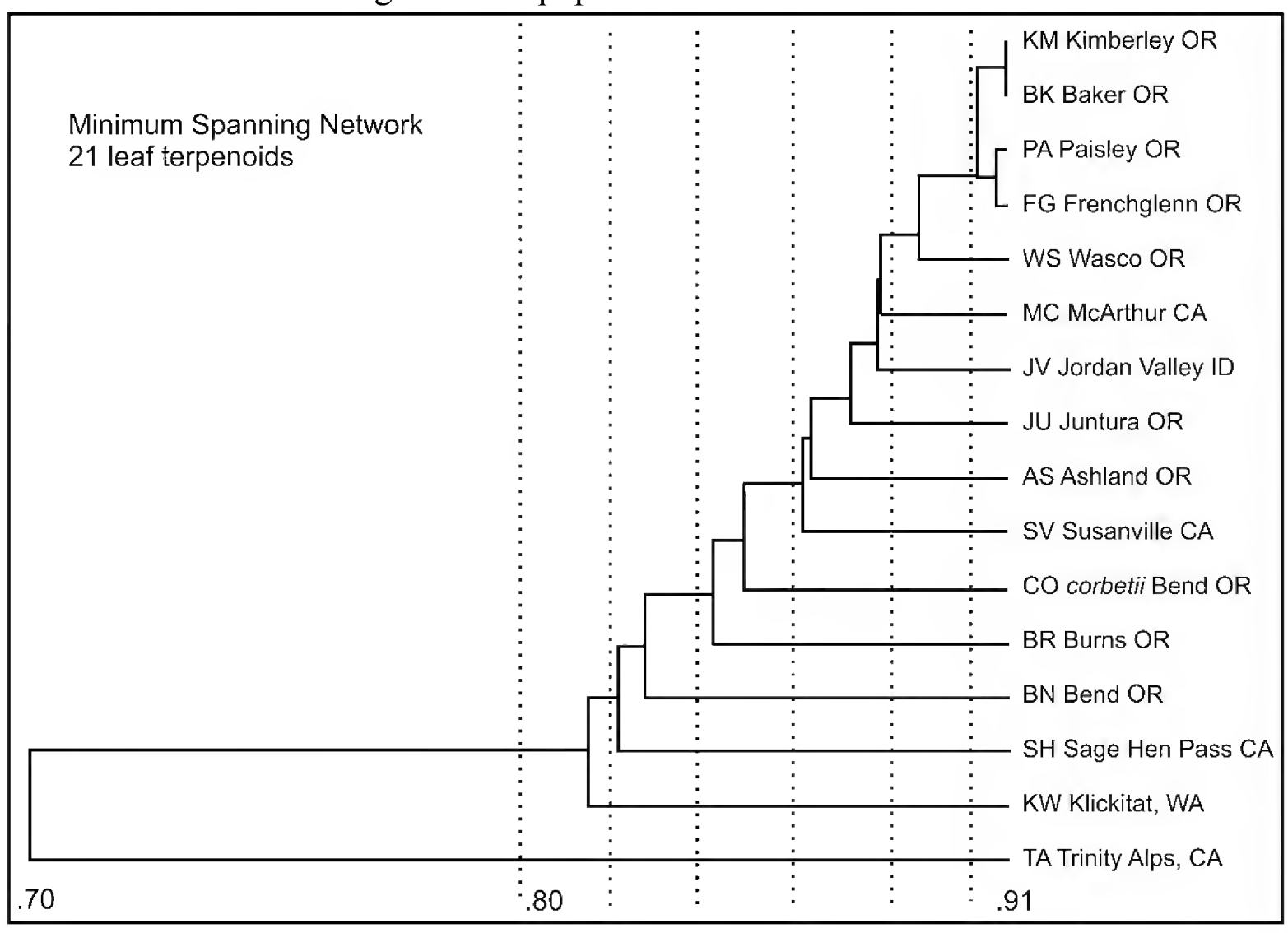

Figure 2. Minimum spanning network based on 21 leaf terpenoids. Notice the divergence of the TA (Trinity Alps, CA) population in its oil. The dotted lines are the contoured levels used in Figure 3.

Contoured clustering shows (Fig. 3) the divergence of the Trinity Alps population, joining last to Burns, OR at an oil similarity of 0.70 (Fig. 3). The leaf oils of the Burns population show some differentiation from adjacent populations BK, KM, JU and FG (Fig. 3, Table 1).

Generally, the leaf oils of *J. occidentalis* are fairly uniform throughout its range in eastern Oregon (Table 1, Fig. 3). Although most of the central populations (KM, BK, PA, FG) are very uniform, there is some differentiation on the periphery of the range: AS, Mc, Sv, SH, BN, and KW.

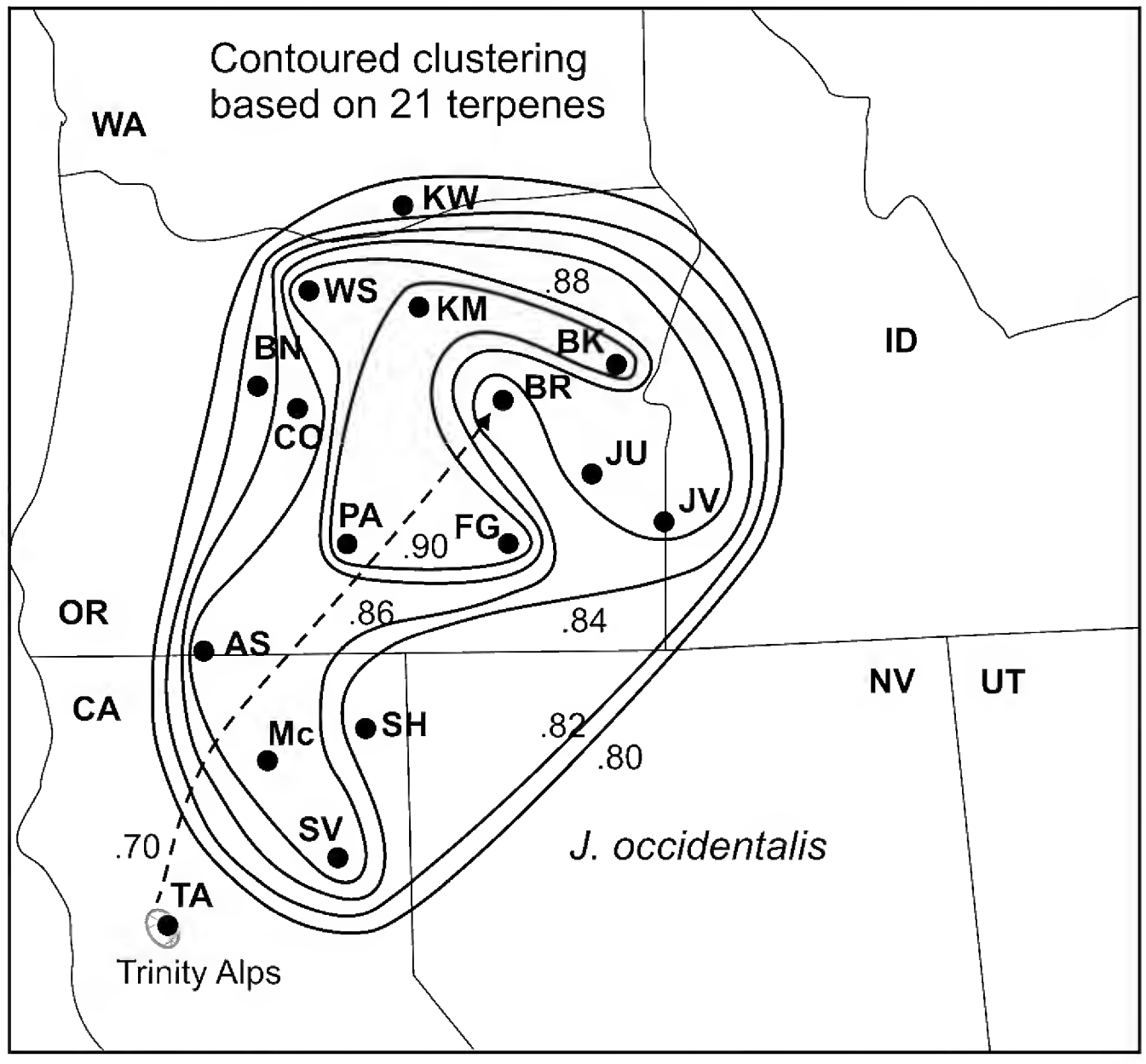

Figure 3. Contoured clustering based on 21 terpenes. See METHODS for population identities.

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Table 1. Leaf essential oil compositions for populations of *J. occidentalis*, Those 21 compounds used in numerical analyses are in boldface. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

AI	Compound	Baker	Bend	*corbetii*	Sage Hen	Burns	Trin Alps
921	tricyclene	0.7	1.1	1.7	1.4	0.8	t
924	α-thujene	0.8	1.0	0.9	0.7	1.0	1.8
932	**α-pinene**	**6.2**	**5.0**	**1.8**	**3.6**	**2.8**	**5.1**
945	α-fenchene	0.3	t	t	t	0.1	-
946	camphene	0.6	1.0	1.2	1.1	0.7	0.3
953	thuja-2,4-diene	t	t	-	t	t	-
961	verbenene	-	-	-	t	-	0.7
969	**sabinene**	**11.6**	**12.0**	**7.4**	**8.2**	**16.9**	**20.4**
974	β-pinene	0.3	0.4	0.2	0.4	0.3	0.7
988	**myrcene**	**1.8**	**1.3**	**1.1**	**1.9**	**2.6**	**3.0**
1001	δ-2-carene	t	t	0.6	0.1	t	0.3
1002	α-phellandrene	0.5	0.8	0.5	1.3	1.2	1.2
1008	**δ-3-carene**	**2.9**	**1.0**	**0.6**	**1.2**	**1.4**	**4.4**
1014	**α-terpinene**	**1.3**	**1.7**	**1.5**	**1.4**	**1.8**	**3.2**
1020	**p-cymene**	**14.3**	**10.7**	**20.0**	**7.1**	**10.6**	**5.5**
1024	**limonene**	**0.6**	**0.9**	**0.7**	**1.4**	**1.3**	**0.7**
1025	**β-phellandrene**	**2.5**	**3.5**	**2.0**	**5.9**	**5.0**	**6.7**
1044	(E)-β-ocimene	0.4	0.1	t	0.2	0.2	0.5
1054	**γ-terpinene**	**2.2**	**3.0**	**2.5**	**2.2**	**3.0**	**5.3**
1065	cis-sabinene hydrate	0.6	0.9	0.4	0.6	0.7	1.2
1086	**terpinolene**	**1.5**	**1.3**	**1.4**	**1.3**	**1.7**	**2.4**
1095	**trans-sabinene hydrate**	**2.5**	**0.7**	**t**	**0.4**	**1.1**	**t**
1095	linalool	t	0.5	1.6	0.7	t	1.5
1112	trans-thujone	0.1	t	t	t	t	-
1118	cis-p-menth-2-en-1-ol	0.6	0.7	0.6	0.8	0.7	1.0

AI	Compound	Baker	Bend	*corbetii*	Sage Hen	Burns	Trin Alps
1136	trans-p-menth-2-en-1-ol	0.8	0.9	0.6	0.8	0.7	0.9
1141	**camphor**	**0.2**	**2.5**	**1.3**	**2.8**	**0.5**	**t**
1145	camphene hydrate	0.2	0.2	t	0.4	0.2	-
1154	sabina ketone	0.6	0.4	0.6	0.3	0.4	0.3
1165	**borneol**	**0.4**	**2.2**	**1.9**	**2.3**	**0.5**	**t**
1166	**coahuilensol**	**1.3**	**0.6**	**0.7**	**1.6**	**1.3**	**2.4**
1174	**terpinen-4-ol**	**5.1**	**6.7**	**6.7**	**5.7**	**5.9**	**9.8**
1179	p-cymen-8-ol	1.4	0.5	1.9	1.1	1.0	0.9
1186	α-terpineol	0.4	0.4	0.3	0.3	0.3	0.5
1195	cis-piperitol	0.3	0.2	t	0.3	0.3	0.1
1207	trans-piperitol	0.3	0.3	t	0.4	0.3	0.5
1219	coahuilensol, me-ether	1.4	1.1	0.6	1.3	1.6	2.7
1238	cumin aldehyde	0.4	0.2	0.3	0.1	0.3	0.7
1249	piperitone	0.4	0.2	0.1	0.5	0.3	0.5
1254	linalool acetate	0.5	0.1	0.4	-	0.4	0.1
1284	**bornyl acetate**	**11.3**	**9.5**	**24.5**	**20.3**	**13.3**	**t**
1298	carvacrol	0.5	0.4	0.3	0.4	0.2	0.7
1322	methyl-geranate	2.5	1.0	0.5	2.0	3.6	0.8
1325	p-mentha-1,4-dien-7-ol	0.3	t	0.3	0.3	0.3	0.1
1345	α-cubebene	t	t	t	t	t	t
1374	α-copaene	0.7	1.0	-	0.7	0.5	0.6
1387	β-bourbonene	t	0.2	t	0.1	t	t
1429	cis-thujopsene	-	0.9	-	-	-	-
1451	trans-muurola-3,5-diene	0.1	0.1	t	t	t	0.1
1465	cis-muurola-4,5-diene	t	0.1	t	0.2	t	t
1468	**pinchotene acetate**	**1.2**	**0.6**	**0.6**	**1.1**	**1.8**	**2.0**
1475	trans-cadina-1(6),4-diene	0.1	0.3	t	0.3	t	t
1478	γ-muurolene	0.3	0.8	0.4	0.3	0.3	0.1
1484	germacrene D	0.2	0.3	t	0.2	0.2	t
1493	trans-muurola-4(14),5-diene	0.3	0.4	t	0.4	0.2	0.7
1493	epi-cubebol	0.2	0.4	t	0.4	0.2	0.4
1500	α-muurolene	0.5	1.1	0.5	0.6	0.4	0.6
1513	**γ-cadinene**	**1.9**	**3.7**	**1.4**	**1.8**	**1.1**	**1.8**
1518	epi-cubebol	0.1	0.4	0.4	0.5	0.5	t
1522	**δ-cadinene**	**2.3**	**4.1**	**1.9**	**1.2**	**1.8**	**2.2**
1533	trans-cadina-1,4-diene	0.2	0.1	-	1.1	t	t
1537	α-cadinene	0.2	0.4	-	0.2	0.1	t
1544	α-calacorene	0.1	0.3	-	0.1	0.6	t
1548	elemol	-	-	0.4	t	-	-
1574	germacrene-D-4-ol	0.6	0.6	t	0.4	0.4	0.5
1586	gleenol	0.1	0.3	t	t	t	t
1607	β-oplopenone	0.3	0.4	t	0.3	0.3	0.4
1618	1,10-di-epi-cubenol	-	0.2	t	t	t	t
1627	1-epi-cubenol	1.2	1.6	0.7	1.4	0.7	1.3
1638	epi-α-cadinol	0.7	1.1	0.5	0.6	0.6	0.4
1638	epi-α-muurolol	0.8	1.2	0.5	0.7	0.6	0.6
1644	α-muurolol	0.3	0.7	t	0.3	0.2	t
1649	β-eudesmol	-	-	0.9	t	0.3	-
1652	α-cadinol	1.0	1.8	1.0	1.1	1.2	0.8
1675	cadalene	0.4	0.3	t	0.2	0.2	t
1987	**manoyl oxide**	**1.7**	**3.2**	**3.0**	**2.1**	**1.0**	**1.0**
2009	epi-13-manoyl oxide	t	t	t	t	t	t

Chlorogalum pomeridianum (D.C.) Kunth ssp. *austrooreganum* Callahan (Asparagaceae), A new subspecies from Jackson County, Oregon, and adjacent Siskiyou County, California

Frank Callahan
PO Box 5531 Central Point, OR 97502 U.S.A.
callahanseeds@gmail.com

ABSTRACT

A new subspecies of *Chlorogalum pomeridianum* (D.C.) Kunth is described as *C. pomeridianum* (de Candolle) Kunth ssp. *austrooreganum* Callahan A suggested common name, "southern Oregon amole," is proposed. Plants are known from throughout Jackson County, Oregon, with fringe populations extending into Siskiyou County, just south of the Oregon/California border. Published on-line www.phytologia.org *Phytologia 97(4):271- 274 (Oct 1, 2015).* ISSN 030319430.

KEY WORDS: *Chlorogalum*, Amole, Asparagaceae

Three other *Chlorogalum* taxa are known to occur within the range of *C. pomeridianum* ssp. *austrooreganum*. *Chlorogalum grandiflorum* has been found at only two locations in Jackson County near Gold Hill; these populations are widely disjunct from its range in the foothills of the Sierra Nevada, California. The other two, *C. angustifolium* and *C. pomeridianum* var. *minus* occur only on ultramafic (serpentine-influenced) soils in southwestern Oregon. The other two *C. pomeridianum* taxa in Jackson County grow in sites separated by elevation. *Chlorogalum pomeridianum* var. *pomeridianum* is found at lower elevations, rarely reaching 600 m (~2000 ft). In contrast, ssp. *austrooreganum* is found at elevations exceeding 1220 m (~4000 ft). Although their elevational ranges overlap (both are found down to 290 m (1000 ft)), there is only one known site where the two taxa grow in close proximity: near the Jackson/Josephine County line along Savage Creek Road off Hwy 99 east of Grants Pass. At this site, Judith Jernstedt, John Erwin and I conducted extensive surveys and found no morphological indication of hybridization between the two taxa. I also attempted to hybridize these two taxa in the nursery without success. For this reason I have chosen to use the rank of subspecies in describing this taxon, rather than variety.

Chlorogalum pomeridianum var. *pomeridianum*

Bulbs 10(20) cm in diameter, bulb coats brown, not membranous, covered with many coarse fibers. Leaves ca. 20-70 long x 3 cm wide margins usually wavy to strongly undulate. Inflorescences: panicles robust, erect, many branched to 2 m height (maximum 3 m), branching pattern symmetrical. Flowers: open in the evening, (vespertine) perianth parts white with purple midvein, spreading to recurved at anthesis, to 35 mm in length, anthers to 2 mm long, style to 15 mm and not exceeding the perianth, perianth and pedicel of equal length. Capsules, short-stipitate, to 7 mm long containing 1-2 seeds/locule. Seeds: rough. Chromosome numbers 2*n*=30, 36.

Chlorogalum pomeridianum (D.C) Kunth ssp. *austrooreganum* Callahan, ssp. nov. Fig. 1.

Type: USA, Oregon: Jackson County, Hidden Valley Ranch, south of Blackwell Hill. N42° 24' 9.6" W123° 0' 23.8", 1700 ft., on ridge east of Harris Gulch, 20 May 2007, *Callahan CPA-HG-2007* (HOLOTYPE: OSC 243400).

The taxon differs from *var. pomeridianum* in that the panicle is not robust, but fragile and narrower in stature, few-branched and rarely to 1 m in height. Leaves ca. 20-40 cm long x 1.5 cm wide, wavy or not. Bulb coats are strictly membranous, white to cream-colored. Seeds are shiny and black. Plants growing in quartzite talus w/*Erythronium multiscapideum* and *E. hendersonii* with an overstory of *Quercus*

garryana, *Q. kelloggii* and *Arctostaphylos viscida*. The seeds are glossy black, bulb coats white membranous, stems fragile, plants not as tall or robust as typical *C. pomeridianum*. $2n = 30$.

It is somewhat surprising that *Chlorogalum pomeridianum* ssp. *austrooreganum* was neither recognized nor collected by any of the early botanists, because it is widely distributed in Jackson County, Oregon, and populations are quite common in the northern-most parts of Siskiyou County, California. *Chlorogalum pomeridianum* var. *pomeridianum* is widespread in California (Jernstedt 2012) and much of southwestern Oregon, as far north as Douglas County. The southernmost range is in northwestern Baja California Norte, Mexico. However, it is strangely absent from most of Jackson County and all of Siskiyou County where it is largely replaced by *Chlorogalum pomeridianum* ssp. *astrooreganum*. In Siskiyou County, California, ssp. *astrooreganum* is found as far south as the Shasta River at the Interstate 5 overpass junction. It is also quite abundant throughout Jackson County in talus and open habitats with heavy clay soils; it has not been found on serpentine soils.

***Chlorogalum pomeridianum* (de Candolle) Kunth var. *minus* Hoover**
Differs from ssp. *austrooreganum* in bulbs with reddish brown membranous bulb coats with few coarse fibers. Leaves: 20-35 cm long x 1.5 cm wide, margins conspicuously wavy. Panicles much shorter, nearly as wide as tall, branching pattern asymmetrical. Seeds: rough. Chromosome number $2n$=30.

The two other species of *Chlorogalum* that have their northernmost range in southwestern Oregon are described below for comparison with the above taxa.

***Chlorogalum angustifolium* Kellogg**
Bulbs to 5 cm diam., tunics reddish-brown, membranous, with few delicate fibers. Leaves to 1 cm wide x 20 cm long, margins generally flat. Inflorescence: to 70 cm, branches upright, pedicels to 3 mm, slender. Flowers: vespertine, perianth parts spreading, not recurving, to 12 mm, oblong, white midvein lime-colored; stamens to 12 mm long, anthers to 3 mm long, yellow, style 4-8 mm long. Fruits: to 3 mm long, chromosome number n=17.
This species is distinguished from all other Oregon species by its narrow leaves, small stature, and small flowers.

***Chlorogalum grandiflorum* Hoover**
Bulbs to 7 cm, tunics reddish to brown, membranous with few delicate fibers. Leaves to 12 mm wide x 40 cm long, undulate margins. Inflorescence: to 100 cm, branches upright, pedicels 2-5 mm long. Flowers: vespertine, perianth parts recurved, to 3 cm long, linear, white with purple midvein, anthers, yellow, style to 28 mm long. Fruits: to 8 mm long. The short pedicels, to 5 mm long, easily separate this species from the *C. pomeridianum*, varieties and subspecies with pedicels to 35 mm long. Grows on mafic-ultramafic derived soils in Oregon.

Hybridization: As noted above, no suspected hybrids between var. *pomeridianum* and ssp. *austrooreganum* have been found. In contrast, hybrids are quite common where the ranges of var. *pomeridianum* and var. *minus* overlap in western Tehama County, California. The bulbs of the intermediate plants exhibit the coarse bulb hairs and an intermediate height panicle. This observation suggests that var. *pomeridianum* and var. *minus* share a closer genetic relationship with each other than with either with ssp. *austrooreganum.*

Additional specimens examined. USA Oregon: Jackson Co.: Rocky Creek, Applegate, 1700 ft., open hillside, Abundance: moderately sparse. Soil type: rocky, dry, clay. Assoc. species *Arctostaphylos*, *Rhus*. 21 June 1959, *R. Lamb SOC17310*. Jackson Cr., W. Jacksonville, 1700 ft. Habitat, open hillside. Abundance spotty. Soil type, rocky, semi-dry. Assoc. species chaparral, foxtail. Remarks: like a camas. 24 June 1961 *Walt Humphrey SOC17309, SOC17308*. All above specimens designated as Paratypes.

Because European settlement in southwestern Oregon in the 1850s, livestock grazing, agriculture, and expanding urbanization have presumably reduced the abundance of ssp. *austrooreganum*. First the pear industry and now the wine industry are major factors in population decline, primarily due to herbicides and cultivation. In many areas of Jackson County ssp. *austrooreganum* can only be found between the fence line and the highway right of ways, having been grazed out of the open grasslands that are reduced to Mediterranean forbs and annual non-native grasses. According to a local rancher, Gene Hansen (1921-1999), most of the foothill grasslands were dominated by "tall fescue" *Festuca californica* in the early part of this century (Hanson, pers. comm., 1990). Overgrazing of these grasslands resulted in the complete removal of the fescue and replacement by exotic annual grasses. *Festuca californica* is a common associate of ssp. *austrooreganum* and the abundance of both taxa has been severely reduced with the introduction of domestic livestock, in addition to herbivory by native fauna. Amole is highly palatable to black-tailed deer, which forage on all above ground parts. Additionally, pocket gophers and ground squirrels dig and consume the bulbs. The reason this taxon does so well in talus and rock outcrops is because pocket gophers and ground squirrels are unable to dig out the bulbs.

Strangely, horses do not eat this plant, as I observed in a horse pasture in which ssp. *austrooreganum* was abundant even with heavy use of other species. Cattle find the leaves and panicle highly palatable; it takes only about three years of grazing to kill the bulb. I have observed that ssp. *austrooreganum* is absent in areas grazed by cattle, whereas it is abundant where the animals are excluded. Presently, *Centaurea solstitialis* dominates most of the grasslands that were formerly habitat for ssp. *austrooreganum*, which have lost their value for grazing due to this unpalatable exotic. There is a very obvious elevation zone that was once prime habitat for this taxon as one climbs into the foothills from Ashland, either on the Dead Indian Memorial Road or Highway 66. These two areas are now dominated with *C. solstitialis* and non-native annual grasses. Fortunately, some large populations of ssp. *austrooreganum* currently exist on Sprignett Butte in northwestern Jackson County. The plants are mostly confined to talus slopes and are found all the way to the summit of the peak at over 1219 m.

After conducting surveys of this taxon throughout its range, it seems very likely that over 95% of its distribution is in Jackson County, Oregon, hence the name *austrooreganum*, referring to southern Oregon.

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Figure 1. Holotype of *C. pomeridianum* (D.C.) Kunth ssp. *austrooreganum* Callahan.

Two New Subspecies of *Calochortus umpquaensis* (Liliaceae) from Southwestern Oregon: *C. umpquaensis* Fredricks ssp. *confertus* Callahan and *C. umpquaensis* Fredericks ssp. *flavicomus* Callahan

Frank T. Callahan
P.O. Box 5531 Central Point, OR 97502 U.S.A.
callahanseeds@gmail.com

ABSTRACT

Two new subspecies are described for *Calochortus umpquaensis* Fredricks: *C. umpquaensis* Fredricks ssp. *confertus* Callahan and *C. umpquaensis* Fredericks ssp. *flavicomus* Callahan. Both subspecies are isolated from the nominate species and both are endemic to soils derived from ultramafic substrates. Published on-line www.phytologia.org *Phytologia 97(4): 275-281 (Oct 1, 2015).* ISSN 030319430.

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Nancy Fredricks (1989) published the name *Calochortus umpquaensis* for an endemic mariposa lily found on serpentine-influenced soils in the Umpqua drainage of southwestern Oregon. She collected the type specimen in Douglas County: "8 km southeast of Rt. 138 on County Rt. 17 (Little River Road), southeast of Glide, T26S R3W Sec. 34, on south facing serpentine slope, elev. 300 m, 3 June 1987, Fredricks 382" (holotype: OSC243401). About 20 years later I examined the as yet unmounted type specimen, which was stored in newspaper. The earliest report of this taxon dates back to the 1950s: Reggie Miller, a botanical collector and founder of the Glide Wildflower Show, discovered it near Peel along Little River Road, in Douglas County, Oregon. She collected specimens of the plant, which she recognized as distinctly different from *Calochortus howellii*, and sent them to an Oregon herbarium, either Oregon State University or the University of Oregon. Unfortunately, they appear to have been lost; she did not receive a response from the herbarium and no trace of her specimens has been found in the herbarium at Oregon State University, which now includes the former University of Oregon herbarium (Ray Godfrey, pers. comm.)

In 1989, I found another population of *Calochortus umpquaensis* while exploring Callahan Meadows, just south of Tiller, Oregon. Callahan Meadows is about 20 miles (32 km) south of the site near Peel where Fredricks collected her type specimen. Ray Godfrey, a Douglas County botanist, took Fredricks to the Callahan Meadows site and she agreed that it was range extension of the species that she was describing. Shortly thereafter, for the benefit of the Tiller Ranger District (Umpqua National Forest), I mapped the entire distribution of the populations that lie south of Tiller. At this time I recognized that these plants were quite different from the plants that Nancy described; they lacked transverse nectary membranes on the adaxial petal surface that were crested with dendritic trichomes, the normal trait for *C. umpquaensis*. In contrast, the plants from Callahan Meadows had a "forest" of long simple trichomes, (hence the specific epithet *confertus*); they also lacked the dense papillose region above the nectary zone that is the norm for typical *C. umpquaensis*. The same year, I began exploring all the other ultramafic sites south of Lane Mountain southwest to Brushy Butte in southern Douglas County. There I discovered yet another distinct *Calochortus* with long yellow trichomes above the nectary, incised nectary membranes that appeared as segmented units and a reduction of papillose trichomes distal from the nectary on the adaxial petal surface. The yellow trichomes are unique to these populations, hence the name *flavicomus*, meaning yellow hairs.

***Calochortus umpquaensis* Fredricks ssp. *umpquaensis*, Umpqua mariposa lily.**
Stems 20-30 cm long, usually not branching. Leaves: basal leaves narrowly lanceolate, to 40 cm long, adaxial surface covered with lineal rows of simple trichomes; cauline leaves 1, not prominent. Inflorescences: 1-5 flowered; bracts 2, subopposite, narrowly lanceolate. Flowers: sepals lanceolate-acuminate, to 2 cm long, petals to 40 mm long, white to cream colored, with dark purple crescent above the nectary, nectary consisting of 3-4 rows of membranes that are not incised nor segmented and crested with dendritic trichomes, adaxial surface strongly papillose, with long simple white trichomes often dendritic at base. Filaments 7 mm long, anthers to 7 mm long. Fruits: nodding, ovate, 3.0-5.5 cm long. Seeds irregular shaped, cream yellow. Chromosome number, $2n$=20 (Fredricks 1989).

***Calochortus umpquaensis* Fredricks ssp. *confertus* Callahan, ssp. nov. Fig. 1.**
TYPE: USA, Oregon: Douglas County, Tiller, Callahan Meadows, 42° 53' 54.48" N, 122° 57' 53.81" W, ca. 823 m., 3 June 1989 *Callahan 534* (HOLOTYPE: OSC 247669; ISOTYPE: SOC).

The Callahan Meadows mariposa lily differs from the nominate species in the following flower traits: adaxial petal surface not strongly papillose, except zone proximal to the nectary (petal claw), nectary membrane absent, and a dense area of long trichomes are present in the nectary zone; petals: blotch-burgundy, not dark purple (Fig. 2).

Plants growing in soils derived from ultramafic substrates in open, dry meadows. Plants common, but scattered, as this area has been subject to overgrazing by cattle in the past. Unauthorized cattle grazing continues despite a Forest Service closure of this meadow to grazing. Jeanette Sientz, Mary Gerritsen and Ron Parsons accompanied me on this field trip.

***Calochortus umpquaensis* Fredricks ssp. *flavicomus* Callahan, ssp. nov. Fig. 3.**
TYPE: USA, Oregon, Douglas County, ridgeline northeast of Brushy Butte to Buck Peak, confined to ultramafic outcrops and below ridge to Lee Creek Rd. Sec. 15, access on BLM 27-4.5 rd. off South Deer Creek Rd., 43° 09' 44.1" N, 123° 08' 09.5" W, 829 m., 7 July 1990. *Callahan CUF-BB-1-90*, (HOLOTYPE: OSC 243401; ISOTYPE: SOC).

Yellow-banded Umpqua mariposa lily differs from the nominate species in these flower traits: adaxial petal surface slightly papillose, nectary with 3-4 rows of transverse membranes crested with dendritic trichomes that are strongly incised and appear as segmented units; area above nectary zone strongly covered with long yellow hairs; Petals: blotch-purple.

Populations confined to T27S Sec. 35, T28S Sec. 1,2,10,15, Associated speciese: *Ceanothus cuneatus*.

All members of the *C.. umpquaensis* complex appear to be strict endemics of soils derived from ultramafic substrates as they have not, to date, been located on any other soil types. Ultramafic soils are uncommon in Douglas County, so there is a natural scarcity of habitat. Land disturbance activities (road building, quarrying and timber plantations) threaten populations with further loss of habitat. A large population of *C. umpquaensis* north of Lane Mountain has been extirpated due to timber plantation practices. Populations deteriorate under herbicide treatments and in low light conditions under a dense forest canopy. *Calochortus* taxa do best in open meadows, often grassland situations, and some prefer very barren soils that support few other plants. *Calochortus* are also impacted by herbivory. Rabbits, non-native turkeys and deer consume the above ground parts of the plants, quail eat the seeds, and gophers and ground squirrels consume the bulbs. Unauthorized livestock on National Forest lands trample and graze the plants. A common member of the chaparral community, *Ceanothus cuneatus*, that is also adapted to poor soils, often grows with the Umpqua mariposa lily.

Calochortus umpquaensis and its three subspecies are related to a southwestern Oregon complex, noted for the unique architecture of rows of trichomes on the adaxial leaf surface. Members of this group also include *C. coxii* and *C. howellii*, the latter bearing an upright capsule while in all other members the capsule is pendent (Godfrey & Callahan 1988). To date, only *C. elegans* has been rarely found to exhibit trichomes on both the adaxial and abaxial leaf surfaces. Yet another interesting feature of these Oregon endemics is that the nectaries fluoresce in ultraviolet light.

The conservation of the southwestern Oregon complex of *Calochortus* serpentine endemics is difficult because populations occur in a variety of land ownerships, including National Forest, BLM, and private holdings. Livestock grazing is a major problem on all of these land ownerships, and over population of deer and rabbits takes a heavy toll on flowering plants and seed crops. Fire suppression has fostered encroachment of meadows by woody plants, further reducing habitat for *Calochortus*. Road building was noted through several of the plant populations, which also promotes a number of weedy species. Based on my extensive field observations, I believe that if land managers and botanists do not take a proactive conservation role, many of these rare serpentine endemics may be lost in the near future. As one of the landowners remarked to me, "having a rare plant on that site restricts our ability to quarry the site for road building." Perhaps we should consider conservation easements to counter the current consumptive use of these habitat areas.

ACKNOWLEDGEMENTS

I thank Stephen Meyers and Richard Halse for reviewing this article and Cindy Roché for editorial assistance.

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Figure 1. Holotype of *Calochortus umpquaensis* Fredricks ssp. *confertus* Callahan

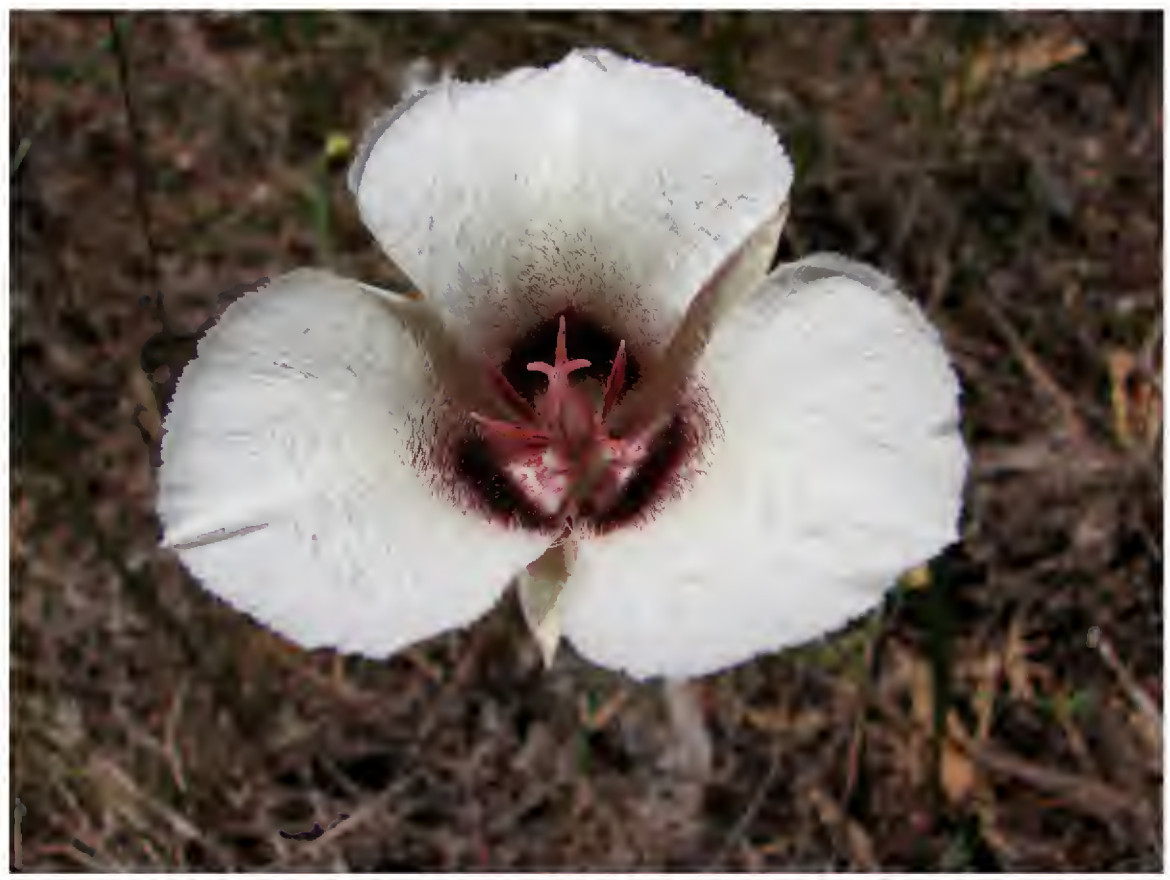

Figure 2. Flower of *Calochortus umpquaensis* ssp. *confertus.* Note the long dense trichomes in the nectary area and the burgundy blotch.

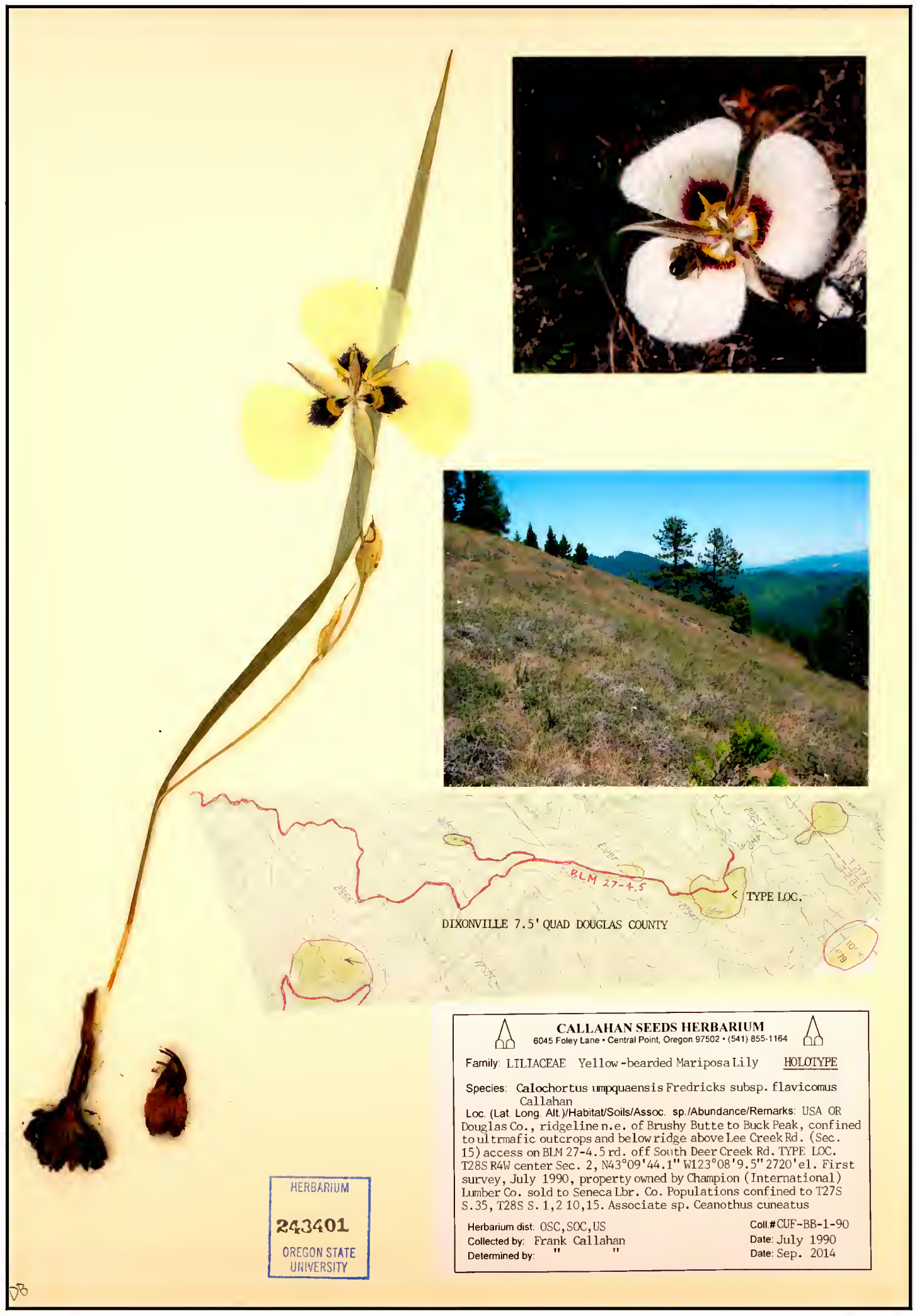

Figure 3. Holotype of *Calochortus umpquaensis* Fredricks ssp. *flavicomus* Callahan

Figure 4. Flower of *Calochortus umpquaensis* ssp. *flavicomus*. Note the long yellow trichomes and the purple blotch.

Calochortus rustvoldii Callahan (Liliaceae), A new species from Los Angeles and Ventura Counties, California

Frank Callahan
PO Box 5531, Central Pt. OR 97502
callahanseeds@gmail.com

ABSTRACT

A new species of *Calochortus*, *C. rustvoldii* Callahan is described from Los Angeles and Ventura counties, California, and a comparison is made with *Calochortus obispoensis* and the *Calochortus C. weedii* complex. Published on-line www.phytologia.org *Phytologia 97(4): 282-285 (Oct 1, 2015).* ISSN 030319430.

KEY WORDS; Liliaceae, *Calochortus*, Placerita Canyon.

On 7 November 2005, while botanizing south of Placerita Canyon Road, Los Angeles County, CA, Ed Rustvold collected some bulbs in steep chaparral terrain east of Santa Clarita. It was well past the flowering season so he was unable to identify the species. As a botanist and grower of geophytes, Rustvold suspected that the bulbs might be *Calochortus weedii* because the site was within the range of that species. The following year, after the plants flowered in his garden, he realized that it might be an undescribed species. This led him to send bulbs to me, in addition to Sean Lake of Navato, California, an avid hybridizer of *Calochortus*.

After growing the bulbs, I compared my specimens with other putatively, closely related species and determined they exhibited intermediate morphology between *Calochortus obispoensis* and yet other taxa within the *C. weedii* complex (*C. fimbriatus*, *C. plummerae*, *C. weedii* and varieties).

The most striking trait shared between Rustvold's specimens and *C. obispoensis* is the perianth, which is somewhat rotate and has flat perianth segments. Conversely, the perianth of *C. weedii* is campanulate (cup shaped), with petals that are broader at the distal end. In addition to the rotate perianth, which is uncommon in the genus, the other obvious feature in common between *C. obispoensis* and *C. rustvoldii* are the sepals which are longer than the petals. The petals of *C. rustvoldii* differ from *C. obispoensis*, however, in that they are broadly wedge-shaped with serrate to fimbriate margins; in contrast, the petals of *C. obispoensis* are much reduced, taper distally to a point, and exhibit very long trichomes along the margin. In commonality, the bulbs of *C. weedii*, *C. obispoensis* and *C. rustvoldii* all share the feature of having coarse reticulate hairs, thus grouping these species in Ownbey's (1940) Section III of Cyclobothera.

On 1 June 2013 Ed Rustvold, Sean Lake and I collected several more bulbs at the site of Rustvold's original discovery. No plants were in flower. As such, my description is based on cultivated plants from the wild-collected bulbs. Rustvold and Lake later collected bulbs off Goodenough Road, north of Fillmore in Ventura County, California. These collections, when later grown were found to be *C. rustvoldii.* There is an approximate air distance of 23 miles, (37 km) between the Goodenough Road and Placerita Canyon Road sites. Several other *Calochortus* species share the same habitat zone, including *C. plummerae*, *C. fimbriatus,* and *C. clavatus* var. *clavatus.*

***Calochortus rustvoldii* Callahan sp. nov. Fig. 1.**
Type: USA, California: Los Angeles Co., Santa Clarita, Placerita Canyon Road, San Gabriel Mountains 34° 22' 25.16" N, 118° 29' 03.04" W, 488 m., 1 June 2013, *Callahan CR-PC-1-2013* (HOLOTYPE: OSC 243403 is from garden grown bulb, collected at given site one year earlier)

Bulbs: coat fibrous-reticulate, 2 cm in diameter. Stems: 30-60 cm, slender, 3-6 branched, bulblets none. Leaf: basal 30 cm long x 1.5-2 cm wide, withering, cauline 2-6 to 7 cm long. Flowers: erect, 3-6, perianth rotate, sepals to 3 cm, narrowly lanceolate, often inrolled, petals to 2-2.5 cm long x 1.3 cm wide, broadly wedge shaped. Petal claw with fine carmine pencilings, nectary yellow, rounded at base with long trichomes tapering to a point. Long yellow trichomes cover 2/3 of the petal surface with a carmine color band on the transverse petal apex with same-colored trichomes. Petal tips serrate/fimbriate. Filaments: 9 mm, dilated at base. Anthers: 9 mm, oblong, brown. Fruits: capsule erect, 6-7 cm x 6 mm. Seeds: flat, net-like surface. Note: carmine colors fade purple on dried herbarium specimen (Fig. 2).

Calochortus rustvoldii differs from *C. obispoensis* in petal shape and trichomes. *Calochortus rustvoldii* has broadly wedge-shaped petals while *C. obisopoensis* has triangular-shaped petals that form a strong point at the petal apex. The petal apex of *C. rustvoldii* is serrate with few fimbriate margins, whereas the petals of *C. obispoensis* bear long trichomes and the non-serrate margins are highly fimbriate, especially near the tip. *Calochortus obispoensis* is the most floriferous of the genus, with counts as high as 100 flowers and bulbs can exceed 3 cm in diameter. *Calochortus rustvoldii* peaks at 6 blooms (in cultivation), with bulbs up to 2 cm in diameter. The filaments are not dilated at the base, 6-7 mm long, and anthers are 3-4 mm long for *C. obispoensis* versus having dilated filaments of equal length and anthers approximately 9 mm long for *C. rustvoldii*.

Calochortus rustvoldii differs from the rest of the *C. weedii complex* with its smaller flowers and rotate perianth. All other members of this complex exhibit strongly campanulate flowers. Both the filaments and anthers in this complex are larger (filaments 12-15 mm long and anthers 8-10 mm long) than those of *C. rustvoldii* (filaments and anthers approximately 9 mm long). My search of all California herbariums (personal visits) showed no collections that conform to *Calochortus rustvoldii*.

ACKNOWLEDGEMENTS

I thank Stephen Meyers and Richard Halse for their review of this article and Cindy Roché for editing. This new *Calochortus* is named in honor of Ed Rustvold, whom has been growing and comparing geophytes in his home nursery in the San Francisco Bay area for many years. It was Ed who first thought this plant might be new to science. I have traveled with Ed Rustvold and Sean Lake on many forays and share their enthusiasm with these beautiful monocots.

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Figure 1. Holotype of *Calochortus rustvoldii*.

Figure 2. *Calochortus rustvoldii* flower. Note the rotate perianth.

nrDNA and petN-psbM sequencing reveals putative *Juniperus oxycedrus* L. from Azerbaijan, Bulgaria, Cyprus and Israel to be *J. deltoides* R. P. Adams

Robert P. Adams
Baylor University, Biology Department, One Bear Place, #97388, Waco, TX 76798, USA,
Robert_Adams@baylor.edu

Vahid Farzaliyev
Deputy Director for Science, Central Botanical Garden, Azerbaijan National Academy of Science, Badamdar shosse 40, Baku, Azerbaijan, AZ 1073.

Salih Gucel
Environmental Research Institute, Near East University, North Nicosia, Cyprus

Hagar V. Leschner
Collection Manager, The Herbarium (HUJ), The National Natural History Collections, The Hebrew University, Edmond J. Safra Campus, Giv'at Ram, Jerusalem 91904 Israel

Tuğrul Mataraci
Tarabya Bayırı Cad., Prof. Sitesi C Blok D:1 Tarabya-Sarıyer, İstanbul, Turkey

Alexander N. Tashev
University of Forestry, Dept. of Dendrology, 10, Kliment Ochridsky Blvd., 1756 Sofia, Bulgaria

and

Andrea E. Schwarzbach
Department of Health and Biomedical Sciences, University of Texas - Rio Grande Valley, Brownsville, TX 78520, USA.

ABSTRACT

A Bayesian analysis based on nrDNA and petN-psbM (combined 2054 bp) of putative *J. deltoides* from Azerbaijan, Bulgaria, Cyprus and Israel confirmed that they are in the clade with *J. deltoides* and well resolved from *J. oxycedrus*. There is some minor variation in *J. deltoides* (only a one or two bp differences in the entire *J. deltoides* clade). This study supports previous studies that show *J. deltoides* occurs from central Italy, eastward though the Mediterreanea to Azerbaijan as it eastern-most location. *Juniperus oxycedrus* appears to be confined to the western Mediterreanea. The taxa can easily be recognized by the deltoid leaf bases and exposed leaf scars on the tips of seed cones of *J. deltoides* versus the tapered leaf bases and smooth seed cone tips of *J. oxycedrus*. Published on-line www.phytologia.org *Phytologia 97(4): 286-290 (Oct 1, 2015)*. ISSN 030319430.

KEY WORDS: *Juniperus deltoides, J. oxycedrus,* SNPs, nrDNA, petN-psbM, plant geography.

In 2004, Adams described a new species, *J. deltoides* R. P. Adams from the eastern Mediterranean that was morphologically similar to *J. oxycedrus* L., except in having deltoid shaped leaves that were broad at the leaf base and with protruding leaf scar bracts on the end of the seed cones. Subsequently, Adams et al. (2005) found large differences in nrDNA sequences confirming the taxonomic decision. Adams et al. (2005) confirmed that trees previously identified as *J. oxycedrus* in Greece, Turkey and central Italy were, in fact, the new species, *J. deltoides*. Additional gene sequencing of nrDNA plus four cp genes confirmed the initial DNA work in the phylogeny of *Juniperus* (Adams and

Schwarzbach, 2013, Adams, 2014). Adams and Schwarzbach (2012) in a study of *Juniperus* sect. *Juniperus* based on nrDNA and five cp regions showed that *J. deltoides* was in a clade with *J. navicularis* and *J. brevifolia*, whereas, *J. oxycedrus* was in a clade with *J. cedrus*, *J. macrocarpa* and *J. maderensis* (Fig. 1).

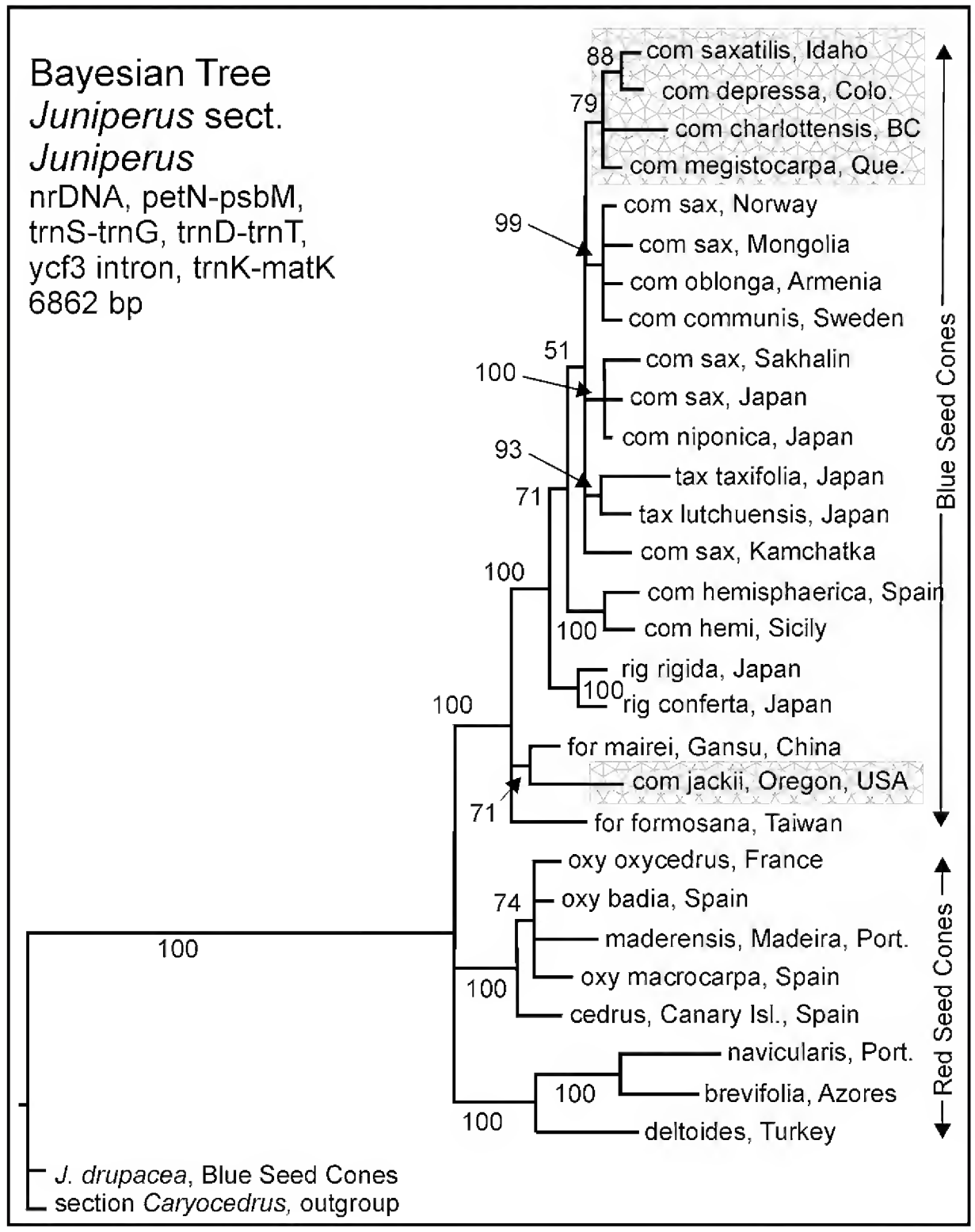

Figure 1. Bayesian tree of *Juniperus* sect. *Juniperus* based on nrDNA and five cp regions. Notice that *J. deltoides* is in a well supported clade with *J. brevifolia* and *J. navicularis*, not in the clade with *J. oxycedrus*, *J. maderensis*, and *J. cedrus*. From Adams and Schwarzbach, 2012.

More recently, *J. oxycedrus* f. *yaltirikiana* Meral, Avci & Ziel., Turkey, has been shown (Adams and Mataraci, 2011) to be *J. deltoides* f. *yaltirikiana* (Meral, Avci & Ziel.) R. P. Adams and *J. oxycedrus* var. *spilinanus* Yalt., Elicin & Terzioglu was proved (Adams et al. 2010) to be *J. deltoides* var. *spilinanus* (Yalt., Elicin & Terzioglu) Terzioglu.

Adams and Tashev (2012) found that the volatile leaf oils of *Juniperus deltoides* in Bulgaria contained key components (cis-p-mentha-2,8-dien-1-ol, carvone, (2E)-decenal, α-copaene, α-copaen-11-ol, α-calacorene, cis-calamenen-10-ol, and cadalene that are absent in *J. oxycedrus* from France; indicating the

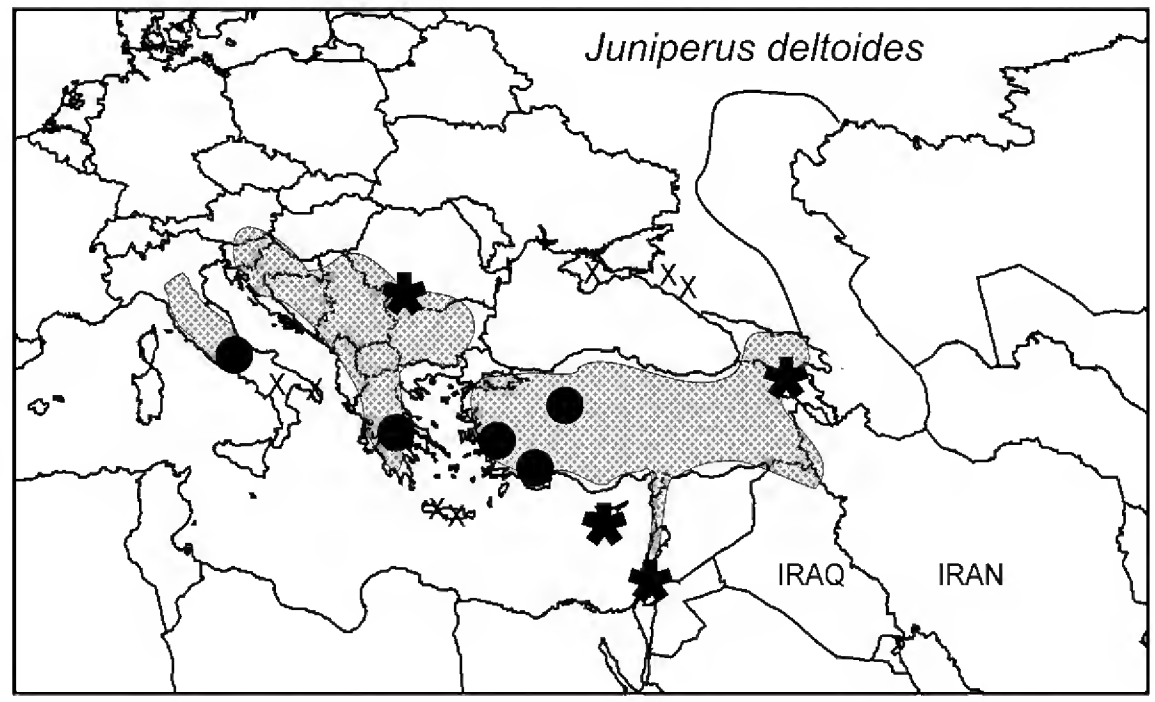

Fig. 2. The distribution of *J. deltoides*. Stars show the locations of newly sampled populations included in this study.

presence of *J. deltoides* in Bulgaria.

The purpose of the present study was to examine additional populations of putative *J. deltoides* in Azerbaijan, Bulgaria, Cyprus and Israel by nrDNA and petN-psbM sequencing to confirm the presence of *J. deltoides* in these regions.

MATERIALS AND METHODS

Plant material*: J. deltoides, Adams 9430-9432*, Turkey; *Adams (Salih Gucel ns) 14544-14547;* Cyprus; *Adams 9436-9438*, Archova, Greece; *Adams 9445-9447*, Riano, Italy; *Adams (Tashev ns) 13126-13130*), Bulgaria; *Adams (Leshner ns) 14463-14465*, Israel; *Adams (Farzaliyev ns) 14466-14468*, Azerbaijan; *J. d.* var. *spilinanus*, *Adams (Mataraci ns) 12064-12066,* Turkey. *J. d.* f. *yaltirikiana*, *Adams (Mataraci ns) 12393-12395,* Zonguldak Prov., Turkey. Voucher specimens are deposited at Baylor University (BAYLU). Vouchers for other locations are reported in previous Adams, et al. papers (cited above).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20^{o} C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

DNA Amplifications and purification: see Adams, Bartel and Price (2009) and Adams and Kauffmann (2010). Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters. Available from http://www.geneious.com/) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v. 3.1 (Ronquist and Huelsenbeck, 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall, 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS1 and ITS2 and flanking regions) gave 1274 bp of data and sequencing petN-psbM (spacer plus flanking regions) yielded 780 bp, for a total of combined of 2054 bp of data. A Bayesian analysis (Fig. 3) shows that all the putative *J. deltoides*, new to this study (Azerbaijan, Bulgaria, Cyprus and Israel), are in the clade with *J. deltoides* and well resolved from *J. oxycedrus*. There is some minor variation in *J. deltoides* (only a one or two bp differences in the entire *J. deltoides* clade).

As previously shown (Fig. 1), *J. deltoides* is part of the *J. navicularis - J. brevifolia - J. deltoides* clade. In contrast, *J. oxycedrus* is part of the *J. oxycedrus - J. macrocarpa - J. maderensis - J. cedrus* clade.

It is interesting to note that colonization of the Azores (*J. brevifolia*) appears to have come from ancestors similar to *J. navicularis* on the continent (Portugal) (Fig. 3). Whereas, colonization of the Canary Islands (*J. cedrus*) likely came from ancestors similar to *J. maderensis* (Madeira) or *J. oxycedrus* (Portugal, Spain or Morocco) (Fig. 3).

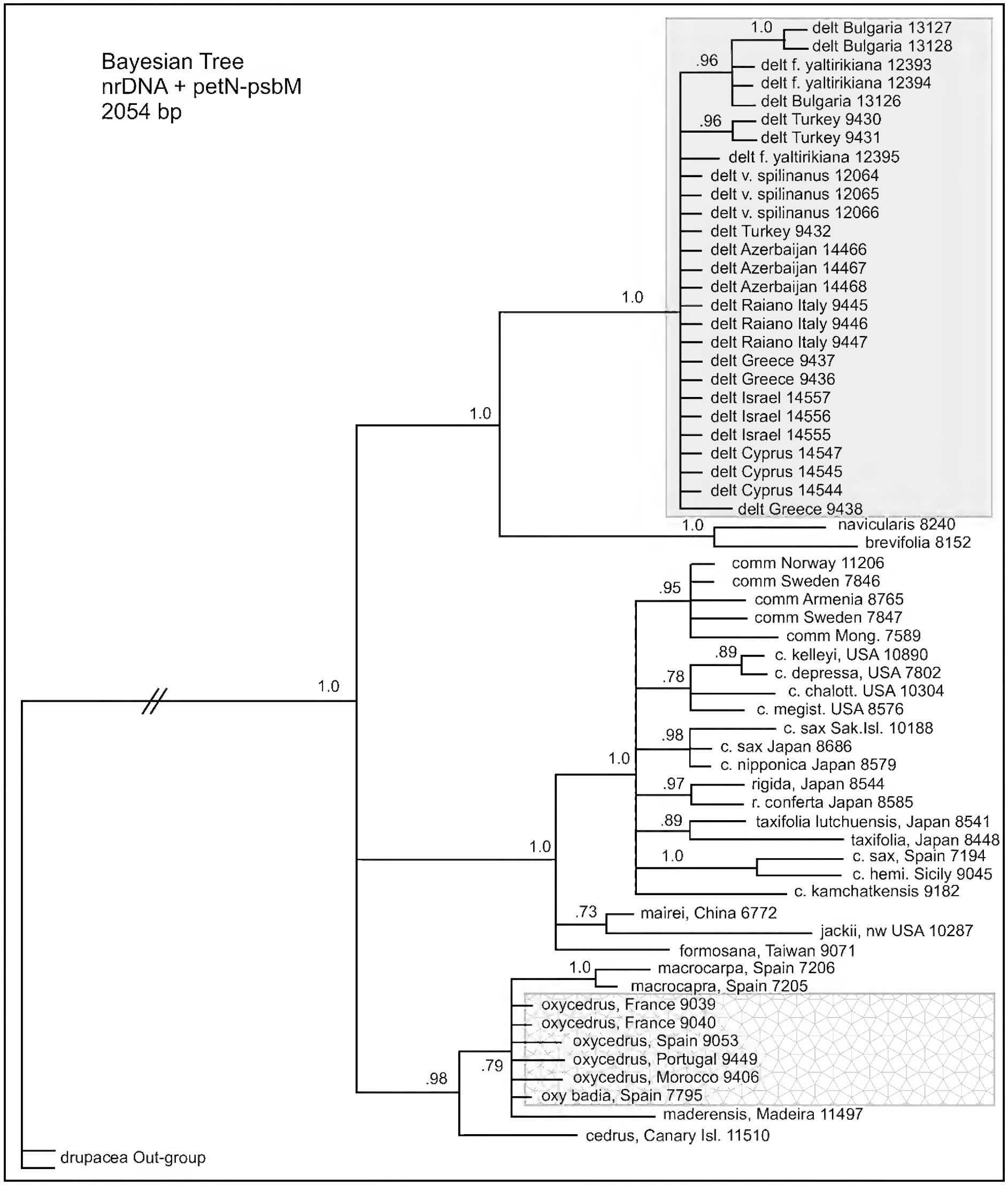

Figure 3. Bayesian tree based on nrDNA and petN-psbM sequences (2054 bp). Numbers at the branch points are posterior probabilities. The shaded area is *J. deltoides* (top right) and the cross hatched area is *J. oxycedrus* (lower right).

ACKNOWLEDGEMENTS

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Nomenclatural Note:

A second attempt for validation of the names *Senegalia x turneri*, *S. x zamudii*, and *Vachellia x ziggyi*

David S. Seigler
Department of Plant Biology, University of Illinois, Urbana, Illinois 61801 seigler@life.illinois.edu

John E. Ebinger
Emeritus Professor of Botany
Eastern Illinois University, Charleston, Illinois 61920

and

Catherine Glass
Pacific Northwest College of Art, Portland, Oregon 97209

KEY WORDS: *Senegalia x turneri* Seigler & Ebinger, nothospecies nov. (*Senegalia berlandieri* x *S. wrightii*), *Senegalia x zamudii* Seigler, Ebinger & Glass, nothospecies nov. (*Senegalia berlandieri x S. reniformis*), *Vachellia x ziggyi* Seigler & Ebinger, nothospecies nov. (*Vachellia collinsii* and *V. pennatula*), name re-validation.

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Recently we published "*Senegalia x turneri* Seigler, Ebinger & Glass" (Seigler, Ebinger & Glass 2012: 447), "*Senegalia x zamudii* Seigler & Ebinger" (Seigler, Ebinger & Glass 2013a: 182) and "*Vachellia x ziggyi* Seigler, Ebinger & Glass" (Seigler & Ebinger 2013b: 298) as new hybrid taxa. The protologue of each taxon (i.e., "*Senegalia x turneri* Seigler, Ebinger & Glass", "*Senegalia x zamudii* Seigler & Ebinger" and "*Vachellia x ziggyi* Seigler, Ebinger & Glass") includes a description in English, listing of the names of the hybrid parents, and holotype citation. Unfortunately, as an act of oversight, for each of these three taxa, we cited the rank of the hybrid taxon as "nothomorph" (= variety; vide Melbourne Code Art. H12.2; McNeill & al. 2012).

Although our intention was to flag the binomials of "*Senegalia x turneri* Seigler, Ebinger & Glass", "*Senegalia x zamudii* Seigler & Ebinger" and "*Vachellia x ziggyi* Seigler, Ebinger & Glass" as "nothospecies", we inadvertently used the misplaced rank term "nothomorph", and thus the names "*Senegalia x turneri* Seigler, Ebinger & Glass", "*Senegalia x zamudii* Seigler, Ebinger, & Glass:, and *Vachellia x ziggyi* Seigler & Ebinger were not validly published (Arts. 5, 37.6).

In a subsequent publication, we attempted to validate the three names (Seigler, Ebinger & Glass 2015). However, our text statement was general ("Seigler, Ebinger & Glass 2012, 2013; Seigler & Ebinger 2013") and was not specifically linked to any one name's description as required by the Melbourne Code Arts. 38.13 and 41.5 (McNeill & al. 2012), and the names for "*Senegalia x turneri* Seigler, Ebinger & Glass", "*Senegalia x zamudii* Seigler & Ebinger" and "*Vachellia x ziggyi* Seigler, Ebinger & Glass" were again not properly validated.

We herewith correct the mistake and again attempt to validate the names "*Senegalia x turneri* Seigler, Ebinger & Glass" (Seigler, Ebinger & Glass 2012, 2015), "*Senegalia x zamudii* Seigler & Ebinger" (Seigler, Ebinger & Glass 2013a, 2015) and "*Vachellia x ziggyi* Seigler, Ebinger & Glass" (Seigler & Ebinger 2013b; Seigler, Ebinger & Glass 2015).

We also erred in using the epithet *zamudii* (honoring the type collector Sergio Zamudio (1953-) and correct it as *zamudioi*

Senegalia x turneri Seigler, Ebinger, and Glass, **nothospecies nov.** (*Senegalia berlandieri* x *S. wrightii*). TYPE: UNITED STATES. TEXAS: Uvalde Co.: Harris Ranch near Cline, 20 miles W of Uvalde on route 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15815* (holotype, ILL).

Senegalia × turneri Seigler, Ebinger & C.E.Glass, Phytologia 94(3): 447. 2012, nom. invalid.

Senegalia x zamudioi Seigler, Ebinger & Glass, **nothospecies nov.** (*Senegalia berlandieri x S. reniformis*). TYPE: MEXICO. Querétaro. Cañón del Río Extorax entre El Plátano y El Timbre, 900 m, 12 Dec. 1999, *S. Zamudio, E. Esparza & E. Zamudio 11241* (holotype, MEXU, photo of holotype at ILL).

Senegalia × zamudioi Seigler, Ebinger & C.E.Glass, Madroño 60(3): 182. 2013 (as "zamudii"), nom. invalid.

Vachellia x ziggyi Seigler & Ebinger, **nothospecies nov.** (*Vachellia collinsii* x *V. pennatula*). TYPE: MEXICO. Oaxaca. A 5 km al NE de San Pedro Tepanatepec, Distr. Juchitán, 200 m, 16 Dec 1978, *M. Sousa, L. Rico & P. Basurto 10157* (holotype: MO).

Vachellia *x* ziggyi Seigler & Ebinger, Phytologia 95(4): 298. 2013, nom. invalid.

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